

ON THROMBOSIS

Aspects of congenital defects in antithrombin III and
vessel wall fibrinolysis

Lilian Tengborn



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Abstract Congenital predisposing factors for deep venous thrombosis (DVT) are presented in this study. About 4000 thrombosis patients from various parts of Sweden have been referred for investigation to the Dept for Coagulation Disorders since 1970. Antithrombin III (AT III) deficiency has been diagnosed in 54 patients (eight families), of whom 37 have had DVT. All of these patients have the so-called classic type of AT III deficiency with a parallel decrease of AT III when assayed both by immunochemical and functional methods. Abnormal variants of AT III are rare and the only family so far diagnosed at Malmö seems to be the first one in the country as a whole. In this family the defect affects various functions of the AT III (progressive thrombin neutralisation, heparin cofactor activity, Xa-inhibition). The treatment of patients with congenital AT III deficiency was studied. Purified AT III concentrate is nowadays available and its <i>in vitro</i> and <i>in vivo</i> properties have been extensively investigated, both those of the original non-heated preparation and of the heat-treated one. The half-life, for example, of the original concentrate was 3.4-4.8 days, but was about 25 % less in the heat-treated form. The half-life was similar in controls, and in both the classic deficiency and the abnormal type of AT III. In AT III deficiency, the administration of concentrate is to be recommended, for instance in conjunction when operations and child-birth. While heparin treatment is controversial in acute DVT, our material included several instances where heparin although decreasing AT III, has adequately prolonged the activated partial thromboplastin time. In this survey is also presented a large family with defective vessel wall fibrinolysis in conjunction with DVT, one of the very first publications of such a defect as a familial phenomenon. A normal tissue plasminogen activator (t-PA) related antigen was demonstrated, but the activity of the recently described t-PA inhibitor was markedly increased.		
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Lilian Tengborn, M.D., Department for Coagulation Disorders, General Hospital, S-214 01 Malmö, Sweden

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ON THROMBOSIS
ASPECTS OF CONGENITAL DEFECTS IN ANTITHROMBIN III AND
VESSEL WALL FIBRINOLYSIS

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Lilian Tengborn
Leg läk, Kr

From the Department for Coagulation Disorders,
University of Lund, General Hospital,
Malmö, Sweden

ON THROMBOSIS
ASPECTS OF CONGENITAL DEFECTS IN ANTITHROMBIN III AND
VESSEL WALL FIBRINOLYSIS

by

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This survey is based on the following publications:

- I Familial antithrombin III deficiency as pathogenesis
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 Johansson L, Hedner U & Nilsson IM
 Acta Med Scand 1978; 204:491-495
- II A Swedish family with abnormal antithrombin III
 Tengborn L, Frohm B, Nilsson L-E & Nilsson IM
 Scand J Haematol 1985; 34:412-416
- III Antithrombin III concentrate: its catabolism in health
 and in antithrombin III deficiency
 Tengborn L, Frohm B, Nilsson L-E & Nilsson IM
 Scand J Clin Lab Invest 1981; 41:469-477
- IV A family with thromboembolic disease associated
 with deficient fibrinolytic activity in vessel wall
 Johansson L, Hedner U & Nilsson IM
 Acta Med Scand 1978; 203:477-480
- V Impaired fibrinolysis. New evidence in relation to
 thrombosis
 Nilsson IM & Tengborn L
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 (ed. J Jespersen, C Kluft & O Korsgaard)
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The articles will be referred to in the text by the Roman numerals above.

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INTRODUCTION

Venous thrombosis is one of the disorders most frequently encountered in general medicine. Although the clinical approach to its prevention and treatment is subject to current pathogenic concepts, its aetiology is still basically that published in 1856 by Virchow, whose famous triad remains applicable today. Thus, the following aspects are of aetiological importance:

- 1) The blood flow (rate of flow, flow volume, turbulence)
- 2) The composition of the blood (platelets, coagulation and fibrinolytic factors as well as their inhibitors)
- 3) The vessel wall (receptors and cofactors of coagulation enzymes on the vascular endothelium, synthesis and release of prostacyclin, factor VIII related antigen, plasminogen activator and its rapid acting inhibitor, wall damage with exposure of subendothelial structures)

AIMS

The aims of this investigation have been:

to study various coagulation factors and vessel wall fibrinolysis in patients referred to the Department for Coagulation Disorders, Malmö, because of deep venous thrombosis, particularly those with a family history of such disease, and with special reference to antithrombin III and the fibrinolytic defence system in vein vessel walls;

to study the characteristics of purified antithrombin III concentrate and its catabolism using ^{125}I antithrombin III;

to evolve guiding principles in congenital antithrombin III deficiency

BACKGROUND TO THE PRESENT INVESTIGATIONS

A) Alterations in the blood flow

Only few aspects of blood flow will be dealt with here. Virchow (1856) stressed the significance of slow-moving blood - no deceleration, no thrombosis. This statement has since been confirmed by researchers using sophisticated modern methods (for instance, involving radionuclides), though others have been unable to verify their findings (review, Bergqvist 1983). Per-operatively, muscle vein velocity is low, and several thrombi developing post-operatively do in fact originate in these veins (review, Bergqvist 1983). In particular, the blood flow is slow in valve pockets, another source of thrombi (Hume et al 1970). Moreover, turbulence in valve pockets probably increases the risk of various blood components (e.g., platelets) being activated, which in turn promotes the development of thrombosis (Smith et al 1972). Although the mechanisms involved in activation of the initial stages of thrombus formation are incompletely understood, there is substantial evidence that human platelets, when exposed to fluid-shear stress in vitro, undergo certain physiological reactions such as platelet aggregation, release of the content of intracellular storage organelles, and platelet lysis (review, Moritz et al 1981).

B) Alterations in the composition of the blood

1. Platelets. General aspects

Platelets have a complicated and manifold function. Following vascular injury, platelets adhere to the exposed collagen or to other subendothelial structures, undergo a change in shape from lentiform discs with smooth surfaces into more spherical forms with protrusions of various shapes, whereupon degranulation occurs and the platelet is able to release substances to induce aggregation (Holmsen 1975, Born 1977, Marcus 1982). Our knowledge of the biochemical events regulating aggregation and degranulation is incomplete, although the release reaction is regarded as one of the most crucial biological mechanisms occurring in vivo. During degranulation ATP (adenosine triphosphate), ADP (adenosine diphosphate), calcium and serotonin are extruded from the dense granules (review, Holmsen & Weiss 1979), and from the alpha-granules are expelled beta-thromboglobulin, platelet factor 4, platelet derived growth factor, fibronectin, fibrinogen, activated factor V, factor VIII related antigen, and factor XIII (Broekman et al 1975, Holmsen & Weiss 1979, Kaplan et al 1979).

Also platelet factor 3 is made available during the release reaction (Sandberg & Andersson 1979). After the release reaction, platelets become sticky on their surfaces permitting other platelets to attach themselves and thus aggregates are formed which are initially reversible. Platelet factor 3 and platelet membranes provide surfaces and receptors for coagulation factors and thereby promote coagulation, resulting in the formation of an irreversible platelet plug.

Beta-thromboglobulin (BTG), first isolated and characterised by Moore et al (1975a), has no apparent biological activity but is regarded as a useful marker of in vivo platelet release reactions.

Platelet factor 4 (PF4), a heparin-neutralising protein but distinct from protamin, was identified by Moore et al (1975b). Owing to its heparin-binding capacity, PF4 is probably of importance in heparin treatment (Niewiarowski 1977).

At the purification process of PF4 a fraction with low affinity for heparin was demonstrated, low affinity PF4 (LAPF4). This factor is closely related to BTG, both proteins sharing common antigens and having similar amino acid sequences. It has been suggested that BTG is a stable proteolytic fragment of LAPF4 (Rucinski et al 1979).

Platelet derived growth factor (PDGF), first characterised by Heldin et al (1981), is a potent stimulator of the proliferation of various cells such as arterial smooth muscle cells. It has been proposed that its activity is responsible for the platelet-vessel wall interaction in the development of arteriosclerosis (Ross & Vogel 1978). It has also been shown that PDGF stimulates the synthesis of prostacyclin (Coughlin et al 1980).

Platelets have enzymatic pathways for metabolising their phospholipid-bound arachidonic acid to the aggregating endoperoxides prostaglandins G_2 and H_2 as well as thromboxane A_2 (TxA_2), which is an extremely potent inducer of platelet aggregation (Hamberg et al 1975) and greatly accelerates the growth of an incipient platelet thrombus. TxA_2 is also a potent vasoconstrictor (review, Moncada & Vane 1978).

As suggested by Walsh (1974), an important platelet function is the provision of a phospholipid (PL) surface for intrinsic coagulation. Miletich et al (1977) demonstrated a specific receptor for activated human factor X (Xa), which becomes available after the release reaction. Bound Xa catalyses prothrombin activation more efficiently than does pure PL surface. It was suggested by

Dahlbäck & Stenflo (1980) that the receptor for Xa consists of activated factor V (Va) and PL. Activated coagulation enzymes bound to the platelets seem to be protected from inactivation by plasma proteinase inhibitors (review, Walsh 1981). The platelet factor 3 (PF3) is quite similar to the platelet membrane, but does not contain any glycoprotein (Sandberg 1979). PF3 is usually defined as the PL-related procoagulant activity of platelets, necessary for the intrinsic coagulation of plasma. It is inactive within the platelet, but is activated during the release reaction when it is released both to the surface and to the plasma.

Clinical aspects

Platelets play an important role not only in the development of arterial thrombosis, in which they are the pre-dominant constituent, but also in venous thrombosis in which the initial white part consists of platelets.

Abnormalities in the above-mentioned platelet factors have not, so far, been demonstrated as being familial phenomena in thrombosis. Defects in the endoperoxides and thromboxanes may play a role in the development of thrombosis, though there are still methodological problems in assaying those components for the routine clinical analysis (Mustard et al 1982).

2) Coagulation system

Clotting factors. General aspects

In the activation of the coagulation cascade, two pathways are traditionally distinguished; the intrinsic, via factor XII in the presence of a surface, and the extrinsic via tissue thromboplastin, a membrane-bound phospholipoprotein, and factor VII (Murano 1978, Davie et al 1979, Prydz 1983)(Fig. 1).

In the intrinsic pathway factor XII is activated to activated factor XII (XIIa) which in turn triggers the cascade of reactions. The complicated process of factor XII activation in the presence of negatively charged surfaces (e.g., glass, collagen, kaolin), high molecular weight kininogen (HMWK)(Fitzgerald factor), and prekallikrein (Fletcher factor) was worked out by Heimark et al (1980). Factor XII is the key enzyme, not only for the contact-activation initiating the intrinsic coagulation reactions, but also for kallikrein-kinin formation, intrinsic fibrinolysis, and, via plasmin, the classical pathway of complement activation (Kaplan 1978, Murano 1978). In the presence of calcium, activa-

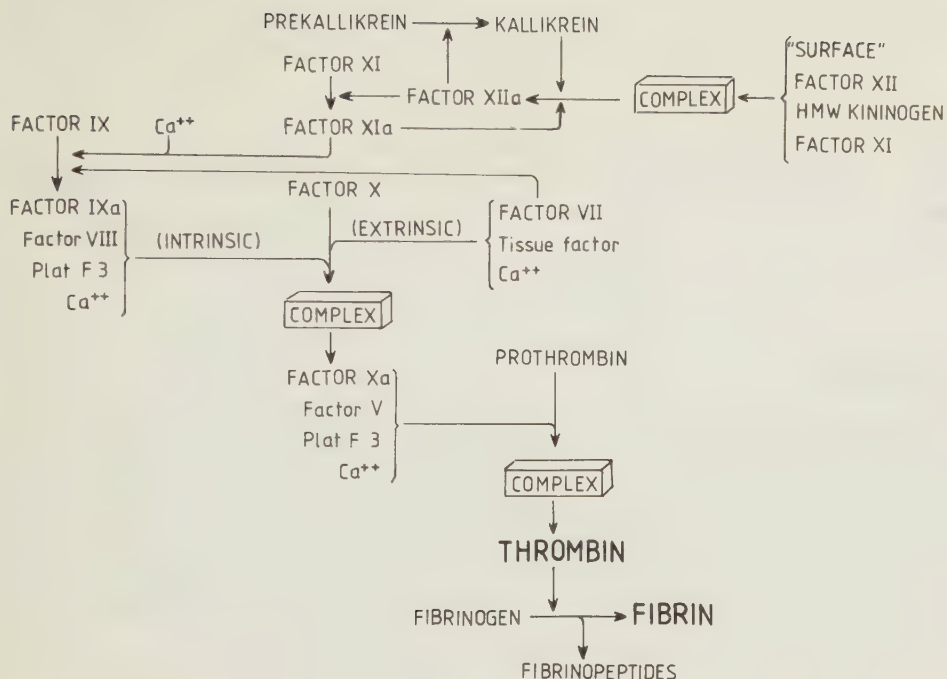


Fig. 1 Coagulation sequence as summarised by Murano (1978).

ted factor XI (XIa), activated by XIIa, converts factor IX to activated factor IX (IXa). With the non-enzymatic accessory protein factor VIII, and in the presence of PL (PF3) and calcium, IXa forms a complex which activates factor X (Xa).

For the function of the vitamin K-dependent proteins (factors IX, VII, X, prothrombin), their γ -carboxyglutamic acids (GLAs) are necessary for the binding of calcium. The binding to PL is mediated via calcium and thus dependent of intact GLAs (Stenflo 1976; review, Prydz 1977).

With another accessory protein, factor V, and together with PL and calcium, Xa forms a complex which activates prothrombin to thrombin. One of the functions of thrombin is to split off two peptides from the fibrinogen molecule thus enabling fibrinogen to be polymerised to fibrin. Thrombin also activates factor XIII thereby inducing an insoluble fibrin clot.

The extrinsic system is activated by tissue thromboplastin, exposed at tissue damage, combining with factor VII and calcium to form an active complex which further activates factor X. Since the conversion of factor VII to activated factor VII (VIIa) is another of the functions of XIIa, factor XII may be considered as a key factor in the activation of both the extrinsic and the intrinsic coagulation systems (Kisiel et al 1977). VIIa, the reaction product of tissue factor, factor VII, and calcium, also functions as an activator of factor IX (Østerud & Rapaport 1977; review, Prydz 1983), thus constituting another example of interaction between the pathways for activation of blood coagulation.

Clinical aspects

Few cases of genetically determined thrombosis with overproduction of coagulation proteins have been described. Gaston (1966) described a family, several members of which had increased factor V content in conjunction with recurrent deep venous thrombosis (DVT). Penick et al (1966) reported on a family with increased factor VIII coagulant activity (VIII:C) and a similar tendency to develop thrombosis.

Congenital dysfibrinogenaemia was reviewed by Mammen (1983) who published a survey of almost one hundred families with functionally abnormal fibrinogen, a few cases in conjunction with thromboembolism (TE).

As already mentioned, the role of factor XII, the Hageman factor, is a multiple one. Inherited deficiency is generally asymptomatic, though several instances of myocardial infarction have been described. Moreover, Mr Hageman himself, the first patient in whom factor XII deficiency was discovered, died of massive pulmonary embolism (PE)(review, Ratnoff 1980).

Surprisingly, a prolonged APTT (activated partial thromboplastin time) has been found in some rare thrombosis patients, namely those with so-called lupus anticoagulants. Lupus anticoagulant is an immunoglobulin (usually IgG, or, more rarely, IgM); the hall-mark of its presence being a prolongation of the clotting time of PL-dependent coagulation assays that can not be corrected by the admixture of normal plasma (Feinstein & Rapaport 1972, Thiagarajan et al 1980). Some patients with lupus anticoagulants have had lupus erythematoses disseminatus or another auto-immune disorder (hence the designation); others have been on such medication as chlorpromazine, hydralazine or procainamide; and still others have had a recent acute infection; but in several cases

no latent causes could be detected (review, Shapiro & Thiaragajan 1982; Boey et al 1983). Familial occurrence of lupus anticoagulant has been described in five cases, though in none of them was there any instance of TE (review, Shapiro & Thiagarajan 1982).

Inhibitors of clotting factors

Antithrombin III. Historical aspects

The presence of a natural inhibitor of blood coagulation was first recognised by Schmidt (1892), and the concept of a blood coagulation inhibitor was further elaborated by Morawitz (1905). Progress in the understanding of coagulation inhibition was made with the discovery of heparin by McLean in 1916. Later it was demonstrated that, in the absence of heparin, the natural inhibitor alone had only a very limited capacity for preventing the conversion of prothrombin to thrombin. Together with heparin, however, this plasma inhibitor not only prevented the formation of thrombin but even inactivated any thrombin that was present (Brinkhous et al 1939). Seegers et al (1942) expanded upon these findings and coined the term heparin cofactor. The slowly progressive inhibition of thrombin in the absence heparin, and its rapid inhibition in the presence of heparin cofactor, have been the subjects of many studies (review, Rosenberg & Damus 1973). Abildgaard (1968) was the first to show that these two functions were indeed related to the same single protein, a finding subsequently confirmed by Rosenberg & Damus (1973). This protein has been termed antithrombin III (AT III), or simply antithrombin. In the slowly progressive inhibition of thrombin, at least two other proteins are involved: α_2 -macroglobulin and α_1 -antitrypsin, each of which accounts for about 25 % of the total slowly progressive activity, while the heparin cofactor activity is confined only to AT III (Lane et al 1975).

Biochemical aspects

AT III is an α_2 -globulin with an M_r of about 57,000–67,000 (Rosenberg & Damus 1973; Miller-Andersson et al 1974; Nordenman et al 1977). It is a single chain glycoprotein composed of 432 amino acids containing 3 disulphide bridges and 4 oligosaccharide side chains (Petersen et al 1979; Chandra et al 1983). The active site of AT III is located at a specific arginine-serine bond in the C-terminal of the molecule (Björk et al 1982). The heparin-binding site seems to be located near the N-terminal (Koide et al 1984), but the heparin-binding is also dependent on the disulphide bond at the C-terminal being intact (Ferguson &

The plasma content of AT III is about 0.15–0.2 g/l (Collen et al 1977, Conard et al 1983). The concentration may also be expressed in units, 1 unit being the amount of AT III present in 1 ml reference plasma.

That AT III is synthesised in the liver was confirmed by cultures of hepatocytes using ^{35}S -methionine (Léon et al 1982); indications have been found in immunofluorescence studies that AT III is synthesised in endothelial cells (Chan & Chan 1979); and immunoreactive AT III has been demonstrated in the microvasculature of the lung, kidney, and large vessel walls (Lee et al 1979).

AT III plays an important physiological role in arresting the coagulation cascade at various stages, by its action as a serine protease inhibitor (Fig. 2). Thrombin and AT III form a stable, inactive, one-to-one molar complex (Abildgaard 1969, Rosenberg & Damus 1973), and other serine proteases besides thrombin (XIIa, XIa, IXa and Xa) may be inhibited by AT III (review, Abildgaard 1981). AT III inactivates IXa, XIa and XIIa more slowly than it does thrombin and Xa.

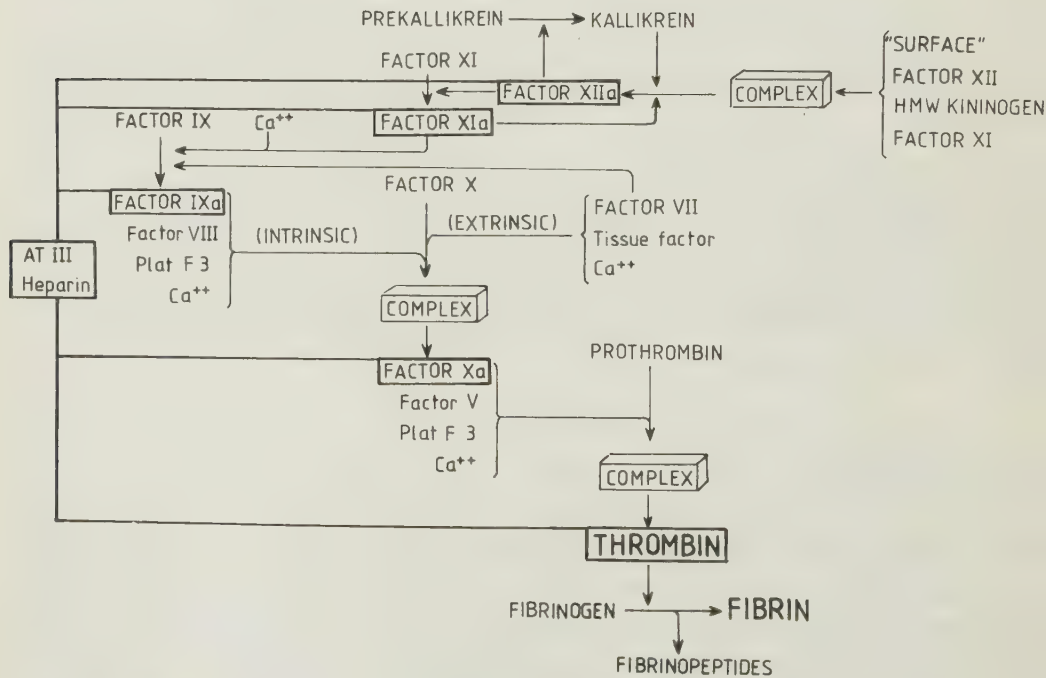


Fig. 2 AT III exerts its inhibiting influence on all stages in the coagulation cascade and heparin accelerates its effect.

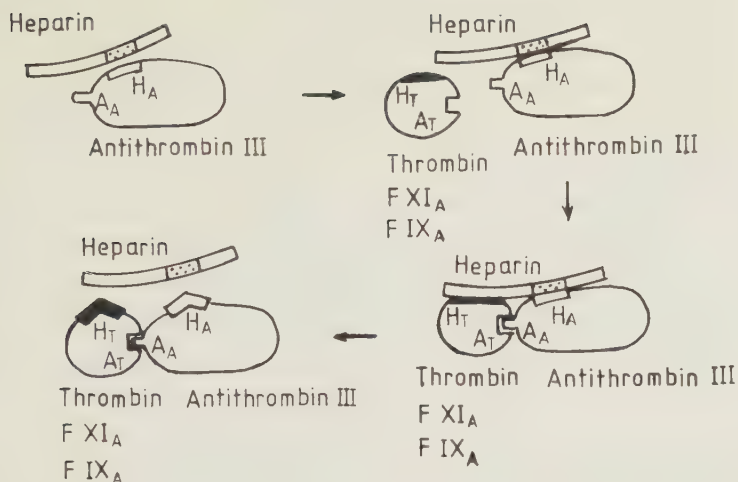


Fig. 3a Tentative mechanism for the inhibition of thrombin, XIa and IXa by AT III in the presence of commercial heparin (Holmer 1981). H = heparin binding site; A = active inhibitor site.

The reactions are generally slow, but in the presence of heparin they are greatly accelerated (review, Abildgaard 1981). Although neither factor VII or VIIa are inhibited by AT III in the absence of heparin, with the addition of heparin, a relatively rapid inactivation of VIIa has been observed (Broze & Majerus 1980). It should be stressed that, as mentioned above, activated coagulation factors bound to platelets seem to be protected against inactivation by AT III.

Plasmin, too, is inhibited by AT III, but the inactivation seems to play a limited role under physiological conditions and at therapeutic levels of heparin (Semeraro et al 1978).

Heparin is crucial to enhance the rate of enzyme inhibition. Three mechanisms have been proposed to account for the activity of heparin. One involves activation of AT III by binding to heparin. A second mechanism operates via enhancement of the reactivity of thrombin with AT III by binding of heparin to the enzyme. A third hypothesis proposes a ternary complex by interaction of both proteins with heparin (review, Hoylaerts et al 1984). Heparin is heterogeneous with respect to molecular weight and affinity for AT III. The heparin used clinically generally has an M_r of 5000–30,000. Only 30–50 % of such heparin has a high affinity for AT III (HA heparin) and displays a high degree of

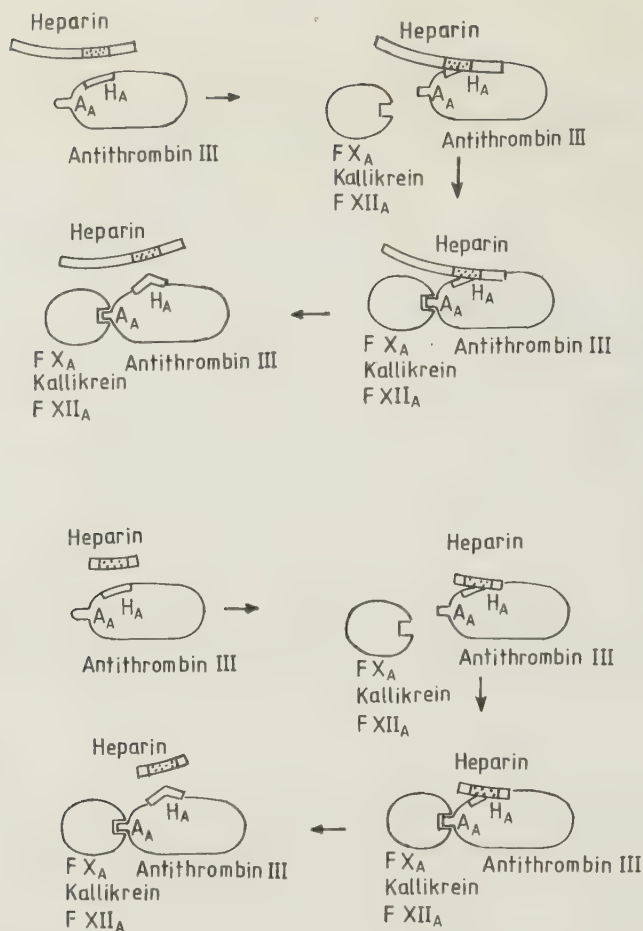


Fig. 3b Tentative mechanism for the inhibition of Xa, kallikrein and XIIa in the presence of commercial heparin and low molecular fragment of heparin according to Holmer (1981).
H = heparin binding site; A = active inhibitor site.

anticoagulant activity (Andersson et al 1976, Höök et al 1976, Lam et al 1976). The anticoagulant effect is dependent upon its molecular weight: with decreasing weight its anticoagulant activity decreases whereas its anti-Xa activity increases (Andersson et al 1976, Laurent et al 1978). The HA heparin contains a unique pentasaccharide sequence which is essential for a strong binding to AT III (Thunberg et al 1982). Holmer and coworkers showed in their studies that HA heparin fragments with an M_r of about 3400, which are almost entirely composed of the AT III binding sequence, neither prolong coagulation time nor inhibit thrombin, IXa and XIa. A molecular weight of more than 4000 was necessary to prolong the coagulation time. Inactivation of Xa, XIIa and kalli-

krein was less dependent on the molecular size of heparin and it was even potentiated by low-molecular-weight heparin that had virtually no effect on thrombin (Holmer et al 1981, 1982). They postulated that for the inactivation of thrombin, IXa and XIa a certain length of the heparin chain was required not only for binding to the AT III but also for a concomitant binding to the enzyme (Fig. 3a). For full potentiation of Xa, XIIa and kallikrein the attachment of AT III alone to heparin seemed to be sufficient (Fig. 3b). It has recently been demonstrated that HA, high molecular weight heparin binds both to AT III and thrombin, thus confirming the hypothesis of a ternary complex for acceleration of the inhibition of thrombin (Hoylaerts et al 1984). It has also been confirmed that the enhancement of Xa inactivation probably not is dependent on a ternary complex formation (Danielsson et al, personal communication).

After its complex formation with AT III, heparin can be released and is thus available to interact with yet another AT III molecule (Jordan et al 1979).

Clinical aspects

Congenital AT III deficiency was first described by Egeberg in 1965, who noted an about 50 % decrease in plasma of progressive AT III and heparin cofactor activity in those members of a large family who suffered from venous TE episodes. Reports of several such families have subsequently been published by, among others, von Kaulla & von Kaulla (1972), van der Meer et al (1973), Marciniak et al (1974), Carvalho & Ellman (1976), Filip et al (1976), and Ambruso et al (1982). In these families a parallel decrease was found in AT III when assayed by immunochemical and functional methods, thus suggesting a decreased synthesis of a normally functioning protein, the so-called classic type, otherwise known as type 1.

Sas et al (1974) published the first report of a family with a thrombotic tendency caused by an abnormal AT III (AT III Budapest). In this family, the AT III related antigen was normal in concentration but the biological functions of the heparin cofactor activity and the progressive antithrombin activity were both decreased (Sas et al 1975, Sørensen et al 1982). Later various types of abnormal AT III were described, see Table I. According to Sakuragawa et al (1983), two main groups can be distinguished; one type in which both the progressive AT III activity and the heparin cofactor activity are reduced despite a normal antigen concentration (e.g., AT III Budapest), and another type, in which only the heparin cofactor activity of AT III is reduced, both the progressive AT III

Table 1

Variants of AT III previously reported.

	Imm- chem	Thrombin inhibition with heparin	Anti Xa activity with heparin	CIE with hepa- rin	Affinity to heparin Sepharose
<u>Type 1</u>					
Egeberg (1965)	L	L	L	N	N
Gomperts et al (1976)	L	L	L	P	
Sas et al (1980)	L	L	L	P	
<u>Type 2</u>					
Sas et al (1975) (Budapest)	N	L	L	P	2 populations
Tran et al (1980) (Basel)	N	L	L		2 populations
Wolf et al (1982) (Paris)	N	L	L		2 populations
Sakuragawa et al (1983) (Toyama)	N	L	L	P	0 (homozygote)
Girolami et al (1983) (Padua 2)	N	L	L	P	N
Sørensen et al (1982) (Aalborg)	N	L	N	N	N
Wolf et al (1985) (Milano)	N	L	L	N	2 populations
Bauer et al (1983a) (Chicago)	N	L	L	N	H

N=normal, L=low, H=high, P=pathological

activity and antigen concentration being normal (e.g., AT III Basel, AT III Paris, AT III Toyama) (Tran et al 1980, Wolf et al 1982, Sakuragawa et al 1983). By analysing not only the progressive activity and heparin cofactor activity, but also anti-Xa activity, still other abnormal types have been identified such as AT III Aalborg (Sørensen et al 1982), which showed decreased heparin cofactor activity and progressive AT III but normal anti-Xa activity in the contrary to e.g. AT III Budapest.

Still another variant, AT III Chicago (Bauer et al 1983a), was found to have a high affinity for heparin in contrast to the low affinity of AT III Budapest, otherwise these two variants showed similar coagulation patterns.

For classification purposes, CIE (crossed immunoelectrophoresis) according to Sas et al (1975) with heparin in the first phase of electrophoresis seems to be necessary in order to distinguish special variants. In AT III Milano (Wolf et al 1985), for example, CIE showed an abnormal pattern of plasma only when heparin was omitted from the first gel dimension; otherwise, its coagulation assays could not be distinguished from that of AT III Budapest.

Also for differentiation of the classic type or type I CIE is important. Gomperts et al (1976), Sas et al (1980), and Leone et al (1983) described one family each with a variant of type 1 with an abnormal pattern in CIE, suggesting the existence not only of a decreased synthesis but also of an abnormal heparin cofactor activity.

AT III deficiency is mostly inherited in an autosomal dominant way. The deficiency is only partial, being about 40–60 % of normal. Often AT III deficient patients have their first attack of TE in their twenties or as teenagers, but even children may be affected. A TE episode generally occurs in conjunction with some other factor predisposing to TE. In most published cases the thrombosis has been located in the venous system though patients with arterial thrombosis have been described (Bjarke et al 1974, Nagy & Lozonczy 1979, Sakuragawa et al 1983).

Various figures have been reported for the prevalence of congenital deficiency. Abildgaard (1979) found five cases with AT III deficiency among 25,000 women. Ødegård et al (1976) found one patient among 396 healthy adults. Rosenberg (1975) estimated the frequency in Massachusetts to be 1:2000, and 2 % of patients who had had DVT. Lechner et al (1977) found 13 patients (belonging to 9 families) among 290 patients with recurrent DVT.

Acquired AT III deficiency. As already mentioned, AT III is synthesised in the liver consequently its concentration in plasma is generally reduced in acute and chronic liver diseases (review, Abildgaard 1981).

In patients with proteinuria, AT III is lost into the urine with other plasma proteins (Kauffman et al 1976), and in nephrotic syndrome the decrease in AT III may be partly responsible for the development of TE (Thaler et al 1978, Kauffman et al 1978).

Decreased AT III is observed in conditions aggravated by an activation of the coagulation system, such as obstetric complications or septic shock (review, Abildgaard 1981).

There have been several reports on the use of oral contraceptives containing oestrogen being accompanied by reduced AT III (Fagerhol & Abildgaard 1970, Ambrus et al 1976). Even contraceptives with a low oestrogen content was by some authors (Conard et al 1980) found to reduce AT III, while others (Hedner & Nilsson 1973, Wessler et al 1976, Sveger 1979) could not demonstrate any decrease. McEntee et al (1981) found a significantly decreased AT III in women on such oral contraceptives, though none of the subjects fell outside the normal range.

Interest has been focused on a pre-operative assessment of AT III to discover whether post-operative TE could be predicted. Sagar et al (1976) reported significantly reduced pre-operative AT III activity in patients who subsequently developed thrombosis following total hip replacement, and similar findings were made by Kruse-Blinkenberg et al (1980) in general surgery patients. However, others have not found the pre-operative assay of AT III to be of any predictive value (review, Fredin et al 1983).

After the administration of heparin there is a decrease in AT III (Blombäck et al 1963, Marciniak & Gockerman 1977, Andersson et al 1984). Using ^{125}I AT III Collen et al (1977) found a significantly shortened plasma half-life and increased fractional catabolic rate during heparin treatment. When patients with normal AT III concentrations are put on long-term heparin prophylaxis, their plasma AT III content initially falls, but later returns to normal despite unchanged or increased dosages of heparin (Hellgren & Nygårds 1981).

There have been some reports of increase of AT III in patients put on oral anticoagulants, though these findings have not been verified by others (review, Manotti et al 1982).

Aspects of treatment in AT III deficiency. Although plasma can be given to correct AT III deficiency, there is a risk of a resultant circulatory overload. The introduction of heparin affinity chromatography (Miller-Andersson et al 1974) simplified the purification of AT III, and such high-purity concentrates are now available for clinical usage. In patients with congenital AT III deficiency, selective correction of the deficiency by means of AT III concentrates is the treatment recommended under such circumstances as operations or deliveries (Hellgren et al 1982, Mannucci et al 1982).

Concerning acquired AT III deficiency, there are some case reports concerning the beneficial effects of AT III concentrates when used in such cases as complications during pregnancy (Büller et al 1980, Laursen et al 1981, Mosvold et al 1982), haemolytic-uraemic syndrome (Brandt et al 1980), and disseminated intravascular coagulation (Jespersen et al 1982).

Protein C and protein S

General aspects

The vitamin K-dependent protein C was first characterised in bovine plasma by Stenflo (1976), and later in human plasma by Kisiel (1979). Thrombomodulin, a recently discovered cell-surface protein receptor, enhances the capacity of thrombin to activate protein C to its activated form (APC) (Esmon et al 1982). As APC has an inhibiting effect on Va and VIII:C (Marlar et al 1980), the accelerators of the coagulation cascade, it has been suggested as playing an important role as a coagulation inhibitor. Moreover, infusion of APC in dogs gave rise to pro-fibrinolytic activity in the blood, presumably by increasing the concentration of circulating plasminogen activator (Comp & Esmon 1981). Thus, protein C may be an important link between the coagulation and fibrinolytic systems. Species differences seem to exist, however, as shown by Colucci et al (1984), who were unable to demonstrate any effect by human APC on the plasminogen activator in monkeys. An inhibitor against APC was proposed by Marlar & Griffin (1980), being later purified from human plasma (Canfield & Kisiel 1982, Suzuki et al 1983).

Protein S, another recently discovered vitamin K-dependent plasma protein, is described, probably in complex with C4b-binding protein, to function as a co-factor for the anticoagulant activity of APC (DiScipio & Davie 1979, Dahlbäck & Stenflo 1981).

Clinical aspects

Like that of AT III, deficiency of protein C is associated with increased risk of developing DVT. Congenital protein C deficiency was first reported from a family study by Griffin et al (1981), who used an immunochemical method, and a few other such families have since been presented (Broekmans et al 1983). Functional methods have subsequently been developed (Francis & Patch 1983, Bertina et al 1984, Comp et al 1984, Sala et al 1984), and the first family with abnormal protein C in conjunction with TE was reported by Bertina et al (1984). Like AT III deficiency, the defect is inherited in an autosomal dominant way, and in heterozygotes the deficiency is partial with a protein C level of about 50 % of normal. So far only one homozygote has been described, manifested by massive venous thrombosis in a neonate whose parents, both with partial protein C deficiency, were first cousins (Seligsohn et al 1984).

Partial deficiency of protein S in familial TE has recently been described in a few cases (Comp & Esmon 1984, Schwartz et al 1984).

3. Fibrinolytic system

Biochemical aspects

The induction of fibrinolytic activity in the blood results ultimately in conversion of the proenzyme plasminogen to the enzymatically active protease plasmin (Fig. 4). Plasminogen consists of a single polypeptide chain with an M_r of about 90,000 (review, Robbins 1981). Native plasminogen or glu-plasminogen has a glutamic acid at the N-terminal, and is easily degraded to lys-plasminogen with a lysine, valine or methionine at the N-terminal (review, Wallén 1977). Unlike glu-plasminogen, lys-plasminogen has a high affinity for fibrin (Wiman & Wallén 1977). After plasminogen activation, plasmin is formed which consists of two fragments: one light (B) chain with an M_r of about 24,000 which contains the active site, and one heavy (A) chain with an M_r of 60,000. Sottrup-Jensen et al (1979) demonstrated that the A chain contained five triple disulphide loops called 'kringles'* (K) 1-5 where lysine binding sites (LBS) were located (Rickli & Otavski 1975) which interact specifically with certain amino acids such as

*) The term 'kringle' is derived from a Swedish word implying twisted loop(s).

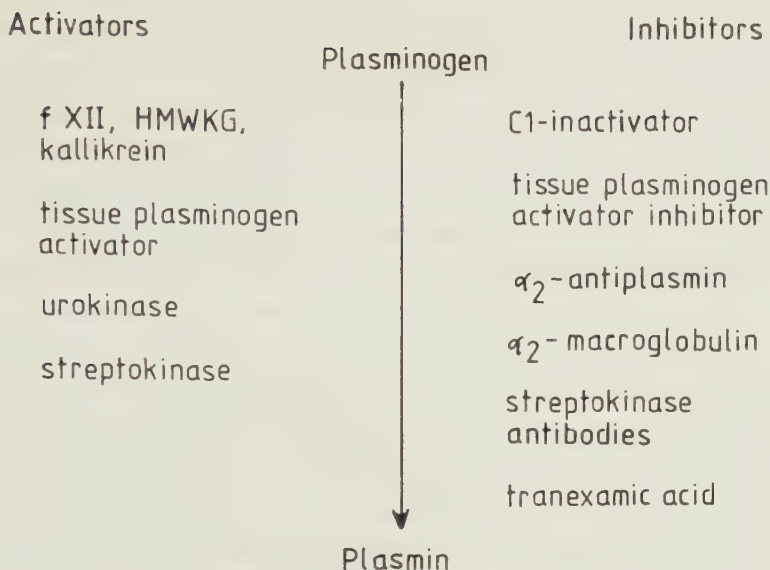


Fig. 4 Activators and inhibitors in fibrinolysis.

lysine and tranexamic acid. LBS could be demonstrated in the two fragments K 1-3 and K 4, but not in the fragment K 5 + B chain. One of the functions of the LBS is to mediate the plasmin-fibrin interaction (Rákóczi et al 1978).

Activators of the fibrinolytic system

As with the coagulation system, the activation of the fibrinolytic system may be divided into intrinsic and extrinsic pathways. Factor XII-dependent fibrinolysis in plasma is known as the intrinsic pathway and requires the presence of a surface. This pathway was recognised already in 1959 by Niewiarowski & Prou-Wartelle, and was later reviewed by Kaplan (1978). In addition to factor XII, prekallikrein, HMWKG, and possibly other components are also involved. From the studies by Kluft et al (1979), it appeared that plasma kallikrein has two functions in the generation of factor XII-dependent fibrinolytic activity; one as a direct plasminogen activator and another as a plasminogen pro-activator.

The extrinsic pathway, via blood, vascular, and tissue plasminogen activator, is thought to be the more important pathway with regard to activation of the fibri-

nolytic system. Plasminogen activators are present in most if not all human tissues (review, Bachmann & Kruithof 1984). Astrup and co-workers (Astrup & Permin 1947, Astrup & Stage 1952) were the first to succeed in partially purifying tissue plasminogen activator (t-PA); later highly purified activator preparations were obtained from hog ovaries (Kok & Astrup 1969), pig heart (Cole & Bachmann 1977, Wallén 1977) and human uterine tissue (Rijken et al 1979, Wallén et al 1981). The t-PA is released into the circulation as vascular plasminogen activator, an activator first described by Aoki & von Kaulla (1971). This activator and t-PA appear to be identical (Thorsen 1977, Wallén 1977; review Bachmann & Kruithof 1984), although it is not yet known whether t-PA undergoes minor biochemical changes during its release.

The t-PA is a serine protease with an M_r of approximately 68,000, and consisting of a single polypeptide chain. After activation by plasmin or trypsin, a two chain molecule is formed, and this cleavage substantially enhances its activating capacity (Wallén et al 1981). Furthermore, the t-PA has been shown to be a relatively poor plasminogen activator in vitro, though in the presence of fibrin its activating capacity was increased dramatically (Wallén 1977, Rånby 1982). Its high affinity for fibrin in conjunction with the fibrin dependent stimulation of t-PA activity, ensures that activation of plasminogen by t-PA is localised to the fibrin of the thrombus and thereby reduces systemic plasminogen activation with degradation of fibrinogen and other plasma proteins (Korninger & Collen 1981). The t-PA was later fully characterised and found to contain two 'kringles' similar to those demonstrated in prothrombin and plasminogen. It was homologous to the human high molecular weight urokinase, plasminogen, and the murine epidermal growth factor (review, Bachmann & Kruithof 1984). The human t-PA cDNA has been expressed in *E.coli* (Edlund et al 1983, Pennica et al 1983).

Of great importance with regard to methodological improvements in the further study of fibrinolytic regulation, were the findings of Rijken & Collen (1981) concerning the characteristics of melanoma cells. From Bowes strain of human melanoma cells in cell cultures, they purified a plasminogen activator which appeared to be immunochemically identical with the activator in human uterus. Although the specific activity was somewhat lower in the melanoma activator than in the uterine, unlike urokinase both of them had a high affinity for fibrin. The t-PA has been isolated in the conditioned medium of endothelial and other normal cells such as trophoblasts and special epithelial cells as well as malignant cell cultures (review, Bachmann & Kruithof 1984).

The t-PA is further described in the section on the vessel wall (p. 27).

Another plasminogen activator, urokinase (UK), has been found in human urine, in the kidney, and in cultures of kidney cells (Macfarlane & Pilling 1947; review, Hedner & Nilsson 1981). UK has been extensively purified (Ball & Day 1970, Holmberg et al 1976); it is now structurally characterised (Henschen & Lottspeich 1983) and occurs in two forms, one two chain form with an M_r of 55,000 and another one with an M_r of 33,000. Immunologically there is no identity between UK and t-PA (Kucinski et al 1968), and of importance is that, unlike t-PA, UK has little affinity for fibrin (Thorsen et al 1972, Wallén 1977, Rijken et al 1979). Recently, a single chain form of UK with an M_r of 55,000 was identified both in human urine and kidney cell cultures (Gurewich et al 1984), and in plasma (Hauert & Bachmann 1984); comparisons of the different forms of UK suggest that this single chain form is a precursor to the other two chain forms and, interestingly, it has affinity to fibrin (Gurewich et al 1984).

Certain neoplasms have been found to release a UK-like plasminogen activator (Rifkin et al 1974, Reich 1975, Åstedt & Holmberg 1976, Camiolo et al 1981, Harvey et al 1982).

The fibrinolytic system may be activated indirectly by streptokinase (SK) (Müllertz & Lassen 1953). SK is a non-enzyme protein with an M_r of 47,000 produced by Lancefield group C strains of β -hemolytic streptococci. SK forms a one-to-one stoichiometric complex with plasminogen or plasmin, thereby converting both the proenzyme and the enzyme into an efficient plasminogen activator (Brogden et al 1973, Castellino 1984). Both UK and SK have been used in thrombolytic therapy, and have been reported to be superior to heparin in preventing from post-thrombotic syndrome (Johansson et al 1979, Arnesen et al 1982) and from disturbances in the pulmonary micro-circulation (Sasahara et al 1975, Sharma et al 1980).

Inhibitors of the fibrinolytic system

In 1975-76 a new specific, fast-reacting plasmin inhibitor in human plasma was described by several groups (review, Collen 1980). This antiplasmin (α_2 AP) is a single chain glycoprotein with an M_r of 70,000, which forms a very stable one-to-one stoichiometric complex with the light (B) chain of plasmin. Free plasmin is supposed to be neutralised immediately in the circulation by α_2 AP. Findings by Aoki et al (1981) suggested that α_2 AP also acted as a plasminogen inhibitor.

α_2 -macroglobulin (α_2 M) reacts more slowly with plasmin than does α_2 AP, and serves as a second line inhibitor (Müllertz & Clemmensen 1976).

Concerning the intrinsic activation of the fibrinolytic system, an inhibitor against XIIa was described by Hedner & Martinsson (1978).

Fast-acting inhibitors of the extrinsic fibrinolysis in plasma were recently demonstrated (Chmielewska et al 1983, Kruithof et al 1984, Verheijen et al 1984). After addition of t-PA the half-life was extremely short, only about one minute. The complex of t-PA and its anti-activator has an M_r of about 110,000, suggesting that the rapid acting inhibitor has an M_r of 40,000 (review, Bachmann & Kruithof 1984). This inhibitor was different from other known inhibitors and was found to react both with t-PA and with UK (Juhan-Vague et al 1984, Kruithof et al 1984, Thorsen & Philips 1984). Using a t-PA inhibitor-free plasma, t-PA and UK formed complexes with α_2 AP and C1-inhibitor but at a low rate (Kruithof et al 1984, Thorsen & Philips 1984) (see further p. 27).

Clinical aspects

Aoki et al (1978) first demonstrated a familial abnormal plasminogen molecule with reduced function in a patient with recurrent TE; the immunochemical concentration of the abnormal plasminogen molecule was normal. Later Robbins (review, 1981) presented various types of abnormal plasminogen, in which after SK activation, abnormal active sites could be demonstrated, and the binding properties of plasminogen to different activators (SK, UK, t-PA) were impaired or abnormal.

Hitherto, no correlation has been demonstrated between high concentrations of the inhibitors α_2 AP and α_2 M and the occurrence of thrombosis.

Defective kaolin-induced, factor XII-dependent fibrinolysis is known to occur in patients deficient in factor XII, prekallikrein, or HMWKG (Niewiarowski & Prou-Wartelle 1959). Whether this so-called intrinsic or factor XII dependent fibrinolysis is significantly involved in the development of TE has yet to be established.

Increased concentrations of inhibitors of plasminogen activation, measured as the capacity to inhibit UK or SK-induced fibrinolysis, have been reported in a few cases with severe TE. Five patients with recurrent idiopathic venous thrombosis, in which two were twin brothers, were reported by Nilsson et al (1961) and

Nilsson (1977); these patients had no demonstrable fibrinolytic activity in the blood and a high content of such inhibitors. Pandolfi et al (1970) reported a case with bilateral occlusion of retinal veins in conjunction with an abnormally high content of the same type of inhibitors. Brakman et al (1966) found a selectively increased inhibition of plasminogen activator in a small group of young men suffering from idiopathic thrombophlebitis and PE. More recently, Alexandre et al (1980) reported a study of two generations of a family with increased concentrations of plasminogen activation inhibitors in those members suffering from recurrent TE.

Clinical aspects concerning increase of t-PA inhibitors is described in the section dealing with alterations in the vessel wall (p. 29).

C. Alterations in the vessel wall

General and clinical aspects

1) Endothelial lining and subendothelial structures

A vessel wall with intact endothelial lining is non-thrombogenic (Spaet & Tsao 1969). Following damage, platelets adhere to the injured wall with great rapidity (Tranzer & Baumgartner 1967, Begent & Born 1970). Adhesion and aggregation play a predominant role in high (i.e., arterial) blood flow rates, while fibrin deposition on subendothelial cells appears to require stagnant blood (Baumgartner 1973). Hedner et al (1973) demonstrated that, compared with those from healthy people, fascia extracts from thrombotic patients occasionally have an increased capacity for aggregating normal platelets.

It has been assumed that thrombi originate either at sites of endothelial disruption or, as already noted (p. 6), in valve pockets. Yet there is substantial evidence that a vein may be free of gross lesions prior to thrombosis (Hume et al 1970), and that a proportion of clinically significant thrombi start in intact soleal veins which have no valves (Nicolaidis et al 1972). Experimental evidence has been obtained suggesting an increased endothelial permeability in veins remote from the site of injury with leucocytes and erythrocytes deposited on intact endothelium (Stewart et al 1978).

2) Cofactors and receptors associated with the endothelial cells

Since even the most feeble stimulus may trigger the coagulation cascade, physiological inhibitors are required to regulate haemostasis. As earlier seen, AT III is the most important inhibitor in circulating blood, although it is slow-acting if heparin is not present. Heparin belongs to the glycosaminoglycans which are sulphated polysaccharides widely distributed in human tissue (Lindahl & Höök 1978). Heparin has not been convincingly demonstrated in blood, but evidence has been found of an endothelial cell surface cofactor responsible for accelerating the activity of AT III (review, Owen 1982). Experimentally, endothelium was cultured on microcarrier beads and used to simulate the in vivo capillary surface. The reaction of AT III with thrombin was enhanced during perfusion of such micro-carrier cultures, suggesting a substance present on the micro-vascular endothelium with the functional properties as a stationary phase cofactor for AT III (Busch & Owen 1982). More recently was even demonstrated that endothelial cells synthesise heparin-like molecules which contain AT III-binding site of heparin (Marcum & Rosenberg 1985).

As already mentioned (p. 19) protein C activation is catalysed by a specific cell-membrane component, thrombomodulin, in complex with thrombin.

Potential clot-promoting activities of endothelial cells have received little attention, but recently it was demonstrated that factors IX and X and their activated forms could bind to endothelial cells. It was found that surface bound IXa retained its coagulant activity and appeared to be even more active as bound than in solution (Heimark & Schwartz 1983, Stern et al 1983).

3) Prostacyclin

Prostacyclin, PGI_2 , synthesised in the endothelial cells of the vessel walls, has been shown to be a potent inhibitor of platelet aggregation (Samuelsson et al 1978), and is probably of importance in providing defence against thrombus formation. Although no case of reduced PGI_2 in conjunction with thrombotic disease has yet been reported, this may only be due to the lack of any simple and reliable PGI_2 assay.

Observations suggesting a relationship between lupus anticoagulants and decreased PGI_2 in patients with recurrent thrombosis are reported but need to be pursued (review, Shapiro & Thiagarajan 1982).

4) Factor VIII related antigen

Since factor VIII related antigen (VIII:Ag), the von Willebrand factor, synthesised in the endothelial cells, is necessary for the adhesion of platelets to the subendothelium (review, Sakariassen et al 1979), it is reasonable to suppose that substantially increased concentrations of VIII:Ag might be of significant importance in causing thrombosis. An increased concentration of VIII:Ag in young patients with stroke (Mettinger 1982), and in patients with DVT (Wahlberg et al 1980) might confirm this hypothesis.

Experimental thrombi from von Willebrand patients show a loose structure without any firm white 'head' composed of platelet aggregates (Åberg & Rausing 1978), and abnormal thrombi with loose structures are more readily dissolved in plasmin (Åberg 1978). These findings may also suggest the importance of VIII:Ag in the development of thrombosis.

5) Activators and inhibitors of vessel wall fibrinolysis

General aspects and methods

The source of the plasma plasminogen activator (PA) has been presumed to be the vascular endothelium. In systems using cultured endothelial cells (ECs) PA activity has been demonstrated (Loskutoff & Edgington 1977, Levin & Loskutoff 1979, Levin 1983) and various molecular forms have been identified of both tissue-type PA and UK-type PA (review, Philips et al 1984). Furthermore, cultured ECs synthesise a fibrinolytic inhibitor that modulates the fibrinolytic activity of the vessel wall (Loskutoff & Edgington 1977, Emeis et al 1983, Levin 1983, Loskutoff et al 1983). Such cultured ECs analysed for PA by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and enzymography on fibrin indicator gels showed predominantly a high molecular weight form, M_r about 100,000, representing an enzyme-inhibitor complex of t-PA M_r 70,000 and a rapid PA inhibitor (Levin 1983, Philips et al 1984). Also t-PA in complexes with C1-esterase inhibitor and α_2 AP were identified (Thorsen & Philips 1984). Moreover, non-endothelial cells of the vascular wall, such as smooth muscle cells and fibroblasts, may generate considerable PA activity (Booyse et al 1981).

As found by Thorsen & Philips (1984) the rapid PA inhibitor in plasma is remarkably similar to the PA from EC cultures. It may be mentioned in this connection that Loskutoff's group recently described a fibrinolytic inhibitor

in human platelets which neutralised the activity both of UK and of t-PA (Erickson et al 1984).

The fibrinolytic activity in the vessel wall has generally been measured using a semi-quantitative fibrinolysis autography technique, as described by Pandolfi et al (1972), which is a modification of the Todd's histochemical fibrin slide technique (1958). Later, Rijken et al (1980) demonstrated that an antiserum against t-PA purified from human uterine tissue quenched the activity responsible for the lysis in the slide technique; antiserum against UK did not affect the activity. Ljungrén et al (1983) demonstrated quenching of the fibrinolytic activity using antibodies against melanoma activator. Both these sets of findings indicate that the activity of the vessel wall is immunologically identical with t-PA.

Noordhoek-Hegt & Brakman (1974) were able to demonstrate inhibitors in the vessel wall using the 'sandwich' technique with the tissue sections incubated under a plasminogen-free fibrin layer followed by application of a fibrinolytically active layer placed on top.

As already noted, there is evidence that the fibrinolytically active endothelial cells continuously release fibrinolytic activity into fluids to which they are exposed. Rapid transient increase of fibrinolytic activity is caused by stress (Cash 1975), as well as by a number of pharmacological compounds such as adrenaline, salbutamol, isoprenaline, nicotinic acid, vasopressin and the synthetic analogue of vasopressin, 1-desamino-8-D-arginine vasopressin (DDAVP) (review, Hedner & Nilsson 1981). Induction of local fibrinolytic activity of the blood by venous obstruction was first studied by Clarke et al (1960). Since spontaneously released fibrinolytic activity is both low and variable, some special method had to be devised to measure it, and Robertson et al (1972), among others, developed a standardised method based on venous occlusion of the arms. The fibrinolytic activity after stimulation by venous occlusion or exercise, or by DDAVP, can be quenched by antiserum against uterine tissue or cultured melanoma cells (Rijken et al 1980, Kluft 1981, Holmberg et al 1982b), thus demonstrating that such stimulation releases a t-PA-related substance to the blood. UK-like material, as measured by RIA (radioimmunochemical assay) (Åstedt et al 1981), was not released, nor was the intrinsic fibrinolytic system affected (Holmberg et al 1982b).

Utilizing the immunoglobulin fraction from antiserum against melanoma activator or uterine t-PA, ELISA (enzyme-linked immunosorbent assays) and IRMA

(immunoradiometric assays) were developed to determine the concentration of t-PA related substance (Holmberg et al 1982a, Rijken et al 1982, Bergsdorf et al 1983).

Functional methods using chromogenic substrate were developed for estimating the activity of t-PA in plasma (Rånby & Wallén 1981, Gyzander et al 1982, Verheijen et al 1984), as well as that of the rapid acting inhibitors (Chmielewska et al 1983, Nilsson et al 1985a).

PAs are not inactivated by SDS, a property exploited by Granelli-Piperno & Reich (1978) who developed a fibrin autograph technique for determining the location of PA activity in slab gels. This approach has been used extensively in characterising the forms of PA produced by EC cultures and PAs present in plasma.

Clinical aspects

Investigating vessel wall fibrinolysis in healthy people, Pandolfi et al (1967) showed that blood fibrinolysis stimulated by venous occlusion was greater in the arms than in the legs, as it was when determined histochemically. Ljungnéer et al (1981) demonstrated that fibrinolytic activity of biopsy specimens from deep major veins and muscle veins was higher than in specimens from superficial veins in the same tract. Several workers have shown that, in conjunction with major surgery, fibrinolytic activity is increased per-operatively, but decreases post-operatively (review, Bergqvist 1983). There are conflicting reports concerning the relationship between decreased pre-operative fibrinolysis and the development of post-operative DVT (review, Bergqvist 1983). Pandolfi et al (1967) demonstrated that vein and blood fibrinolytic activity was lower in patients with thrombosis than in normals. Among patients with recurrent idiopathic DVT and superficial thrombophlebitis, when examined on average three months after an acute episode, fibrinolytic activity after venous occlusion was found to be deficient in 38 %, activator content reduced in 55 %; altogether the vessel wall fibrinolysis was defective in 73 % (Isacson & Nilsson 1972). Using the same methods, a defective fibrinolysis was also found (Sundqvist et al 1981) in a large proportion of patients with axillary vein thrombosis (49 %), and this was the case in young patients with cerebro-vascular ischaemia (Tengborn et al 1981, Mettinger & Egberg 1982).

Using the recently developed methods, the fast-acting inhibitor of t-PA was found to be considerably increased among severely ill patients such as in liver

diseases, pancreatitis, malignancies, septicaemia, and following major surgery (Juhan-Vague et al 1984; Colucci et al 1985). Furthermore, the inhibitor increases successively during pregnancy as reported by Wiman et al (1984) and Åstedt et al (1984). Nilsson et al (1983) found this t-PA inhibitor to be increased among patients with DVT and TIA (transient ischaemic attacks), and in DVT patients the inhibitor was found to be increased more frequently than the synthesis or release of plasminogen activator was decreased (Haglund et al 1984; Nilsson et al 1985b).

Thus, reduced fibrinolytic activity seems to be a fairly frequent finding in various conditions and various groups of patients who have had thrombosis, though only a few workers have reported on the defect as a familial phenomenon. Andersen (1976) reported on a family with arterial thrombosis together with hyperlipidaemia and impaired capacity for releasing fibrinolytic activity from the vessel wall. Jørgensen et al (1982) and Stead et al (1983) described one family each with a high frequency of venous thrombosis in conjunction with reduced fibrinolysis.

LABORATORY METHODS AND CLINICAL MATERIAL

For blood sampling, 5 ml vacutainer tubes were used containing 0.5 ml 0.13 M/l trisodium citrate (1 part of citrate + 9 parts of blood). Platelet-poor plasma was separated as soon as possible after blood collection by centrifugation for 10 min at approximately 2000 g. Fresh plasma was used for the fibrin plate assay, but for the other assays the plasma was stored in aliquots at -20° or -70°C until analysed. For the analysis of t-PA by an amidolytic method, after collection blood was immediately chilled on ice and centrifuged at $+4^{\circ}\text{C}$ for 10 min at 2000 g. For the analysis of t-PA-related substance by IRMA blood was drawn in vacutainer tubes containing 0.05 ml 0.38 M/l (17 %) EDTA.

Pooled plasma was obtained by mixing plasma from 20 apparently healthy donors aged 20-60 years (men and women in equal numbers).

The reference values are given in the various papers.

Coagulation and fibrinolytic assays

Platelet adhesiveness was analysed essentially according to Hellem (1960) using citrated whole blood.

APTT was determined manually using a reagent from General Diagnostics, USA, in accordance with the manufacturer's recommendations.

Factors XII, XI and VIII:C were measured in a one-stage recalcification system (Nilsson 1974).

VIII:R:Ag was measured using Laurell's electroimmunoassay (EIA, Laurell 1972). The specific antiserum was prepared according to Holmberg & Nilsson (1973).

P&P (prothrombin, proconvertin, factor X), factor V, fibrinogen (syneresis method), thrombin time, and reptilase time were measured as described by Nilsson (1974).

AT III was assayed a) with EIA using antiserum raised in rabbits and prepared according to McKay (1981), highly purified AT III according to Miller-Andersson et al (1974) being used for the immunisation; b) as heparin cofactor using the chromogenic substrate S-2238 and bovine thrombin (KabiVitrum AB, Sweden) according to Abildgaard et al (1977); c) with regard to the progressive activity by means of a clotting method using heat defibrinated plasma, as described by Abildgaard et al (1970); d) as the progressive activity by amidolytic method using S-2238 (KabiVitrum AB, Sweden) as described by Bauer et al (1983b); e and f) as anti-Xa activity measured in the presence (e) or absence (f) of heparin as described by Ødegård et al (1976) using the chromogenic substrate S-2222 (KabiVitrum AB, Sweden); g) with CIE carried out according to Sas et al (1975) using heparin (KabiVitrum AB, Sweden) in a concentration of 17 IU per ml agarose in the first electrophoresis and specific antiserum against AT III in the second dimension. As standard for AT III was used the 1st (1978) International Reference Preparation for Antithrombin III, Human, 72/1, NIBSC (National Institute of Biological Science). Using this standard the results were expressed in IU per ml. The results were also expressed as percentage of the mixed plasma of the reference population.

SDS-PAGE was performed in 10 % gel according to Weber & Osborn (1969).

Protein C was analysed with a counter current electrophoresis as descri-

bed by Crowle (1973) using antiserum raised in rabbits kindly supplied by Professor J. Stenflo (Malmö, Sweden).

Plasminogen was assayed with a) single radial immunodiffusion according to Mancini et al (1965), using antiserum raised in rabbits which was provided by the Department of Clinical Chemistry, Malmö, Sweden, and b) an amidolytical method using the chromogenic substrate S-2251 (KabiVitrum AB, Sweden) as described by Friberger et al (1978).

α_2 M was measured with an esterolytic method (Nilsson 1974); α_2 AP with a) EIA using antiserum raised in rabbits (a gift from Dr B. Wiman, Stockholm, Sweden); and b) amidolytical method using the chromogenic substrate S-2251 (KabiVitrum AB, Sweden) according to Teger-Nilsson et al (1977).

Inhibitors of plasminogen activation (UK inhibitors in a clot lysis system) were measured according to Paraskevas et al (1962).

The fibrinolytic activity in the vein walls was measured using Pandolfi's modification of Todd's fibrinolysis autography technique originally described in 1958. Cryostate sections of a vessel specimen were collected in a glass slide and covered with a fibrin film rich in plasminogen. After incubation and staining, fibrinolytic zones appear and by estimating the size of lysed areas the activity was measured semiquantitatively and expressed in arbitrary units (Pandolfi et al 1972). Fibrinolytic activity was quenched according to Ljungnér et al (1983) using the immunoglobulin fraction from antiserum against melanoma cell activator raised in a goat (Holmberg et al 1982a). An immunoglobulin preparation from a normal goat and from antiserum against UK (Åstedt & Holmberg 1976) were used as controls.

Fibrinolytic activity was determined after venous occlusion of the arms and in some cases after injection of DDAVP (Gader et al 1973). The venous occlusion test according to Robertson et al (1972) was performed by applying a sphygmomanometer cuff around each arm and inflating the cuffs to a pressure midway between the systolic and diastolic pressures which was maintained for 20 min. Blood samples were drawn immediately before the cuffs were deflated. The mean increase in fibrinolytic activity in the samples from each arm was calculated. The test was performed on two consecutive days.

The fibrinolytic activity was determined by three different methods: a) fibrin

plate method), b) t-PA S-2251 and c) t-PA IRMA. a) Plasminogen-rich human fibrinogen was used in the plate method as described by Nilsson & Olow (1962), both citrated plasma and resuspended euglobulin (pH 5.9) precipitate being assayed. b) t-PA was measured amidolytically according to Gyzander et al (1983), using lysine-Sepharose, poly-D-lysine and the chromogenic substrate S-2251. c) t-PA related substance was analysed by IRMA as described by Holmberg et al (1982a), using purified antibodies of melanoma activator radiolabelled with ^{125}I by the lactoperoxidase method (Thorell & Johansson 1971). For (b) and (c) melanoma activator was used as a reference, results being expressed in ng/ml.

The fast-acting inhibitor of t-PA was assayed by determining the amount of added purified melanoma activator (LBK 66, KabiVitrum AB, Sweden, 1 ng contains 1 U t-PA) which was inactivated by the plasma. After incubation, residual activator activity was measured using S-2251 as described above. Inhibitor activity was expressed in units, one inhibitor unit being defined as the amount required to neutralise one unit of t-PA. The t-PA inhibitor was only determined in pre-occlusion samples.

The t-PA and its inhibitor were further studied using 6 % SDS-PAGE (Weber & Osborn 1969), followed by zymographic analysis according to Granelli-Piperno & Reich (1978) and Nilsson et al (1985a).

Catabolic studies of AT III concentrate

The original Swedish AT III concentrate was prepared by affinity chromatography on heparin Sepharose gel according to Miller-Andersson et al (1974).

Later the purification procedure was slightly modified as reported by the manufacturer. Moreover, the solution of this preparation was heat-treated for 10 h at 60°C. The concentrates were generously provided by KabiVitrum AB, Sweden.

The radiolabelling procedure was carried out as described by McFarlane (1964) and Glover et al (1967), using ^{125}I -monochloride; an AT III preparation without HSA (human serum albumin) being used for radiolabelling. Labelled AT III was purified on a Sephadex G-50 Fine Column (Pharmacia Fine Chemicals AB, Sweden) which was eluted with saline and the protein peak fractions were pooled. HSA was added in amounts equivalent to those in the commercial HSA-

containing concentrate. The amount of non-TCA (non-trichloroacetic acid) precipitate lable after the sterile filtration varied between 1 and 2 %.

Clinical material

Patients with thrombosis, most commonly in the venous system, referred to the Department for Coagulation Disorders in Malmö. Detailed information is given in the individual papers.

SUMMARY OF THE PRESENT INVESTIGATIONS

The results are only summarised here. The details are given in each paper (I-V).

Familial antithrombin III deficiency as pathogenesis of deep venous thrombosis

Paper I

The largest Swedish family with selective AT III deficiency so far encountered is presented in this paper. At 13 years of age, the propositus had had pneumonia and developed clinical symptoms of DVT in the left leg. Her first pregnancy at the age of 24 was complicated by DVT in the same leg and signs of recurrent TE. When her second child was born three years later, both pregnancy and delivery were free from complications. Ten years later she again had thrombosis in the left leg, phlebographically confirmed and recurring after six weeks, since when she has been on continuous warfarin medication and no more DVT have developed. Altogether, 44 family members in two generations were investigated, of whom 18 were found to have reduced AT III. Of these 18 members, six had had DVT. Yet another member of the family had had DVT which occurred during pregnancy, though her AT III concentration was normal. DVT has usually occurred in conjunction with other factors known to predispose to its development.

The AT III was measured both immunochemically and functionally; the function was analysed as the progressive AT III by a clotting method using heat-defibrinated plasma (Abildgaard et al 1970). The AT III was about 50 % of normal, according to either method. Subsequent amidolytic assessment of the heparin cofactor activity and the anti-Xa activity, both with and without heparin, showed a decrease to about the same level. The pattern obtained by CIE, with heparin included in the agarose gel in the first electrophoretic run, was normal except that peaks were less pronounced. Thus, this family seems to belong to the classic type - i.e., quantitative deficiency of a functionally normal AT III.

Addendum I (see Tables II and III, pp 36-44).

Addendum I

Table II. Brief clinical data on patients with a history of DVT, diagnosed at the Department for Coagulation Disorders as having the classic type of AT III deficiency.
*) denotes in addition defective vessel wall fibrinolysis

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
Family no. 1					
♂ b. 1916	1951 1979	DVT right leg " left leg	Long journey by car Subarachnoidal bleeding+op.aneurysm	No	Ulcers right leg 1974 ligation of insuff. communicant veins+stripping - no prophylaxis
♀ b. 1919	1935 1937-1955 1974 1975	DVT bilat. Thrombophlebitis Thrombophlebitis "	Measles+pneumonia+peritonitis At each of her five pregnancies	Since 1975	Leg ulcers bilat.
♂ b. 1929	1974 1984	DVT left leg Thrombophlebitis right leg	Trauma	No	Ulcers right leg 1984 hernia op. - AT III conc.
♀ b. 1929	1958 1974-1976	DVT left leg Recurrent thrombophlebitis	Pregnancy - third trimester	Since 1984 (VOC mitrale + fibrillatio auricularis)	Pregnancy and delivery 1951, 1956 - no prophylaxis Leg ulcers bilat.
♀ b. 1937	1950 1960 1961	DVT left leg " right leg Thrombophlebitis migrans+ suspect PE	Bilateral bronchopneumonia Pregnancy wk. 22 The same pregnancy wk. 38		Pregnancy and delivery 1964 - no prophylaxis

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♂ b.1941	1974	DVT left leg+PE	Long journey by car	Since 1974	
	1968	Thrombophlebitis migrans left leg	Slight infection + watching TV several hours	Since 1976	1964 appendectomy - no prophylaxis
♂ b.1958	1978	DVT left leg	Pneumonia		
	1980	Thrombophlebitis left leg + suspect DVT		Since 1980	
♂ b.1963	1978	DVT left leg	Myocarditis	Since 1978	
Family no. 2					
♂ b.1925*	1954	DVT left leg	No		1955 stripping bilat.
	1967	Thrombosis mesenteric vein		Since 1967	1956 hernia op. - no prophylaxis
	later	DVT bilat. + thrombophlebitis bilat. several times			1959 cholecystectomy - no prophylaxis, stripping bilat.
♂ b.1930	1965	DVT right leg+PE	No		1961 ligation of insuff. communicant veins left leg
	1974	" " "			1977 sclerotherapy left leg
	1976	" " "		Since 1977	Ulcers right leg
♀ b.1932	1952	DVT left leg	Pregnancy wk.10		
	1984	" right leg + PE	Bronchitis	Since 1984	1974 stripping left leg - no prophylaxis
♀ b.1949*	1967	DVT left leg	Combined contraceptive pills for 3 months		1979 (Feb.) abrasion muc.uteri - dextran prophylaxis
	1979 (May)	" " "	Laparoscopy (March) (heparin + dextran)	Since 1979	1980 hysterectomy - dextran prophylaxis. Leg ulcers left

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♀ b. 1954*	1970	DVT left leg	Pregnancy wk. 10	Since 1970	1970 evacuatio - dextran prophylaxis
"	"	" right leg + PE	Evacuatio		Since 1970 epilepsy
1974	1974	" left leg	"		1974 evacuatio - dextran prophylaxis
					1982 evacuatio - AT III conc. + dextran prophylaxis. Leg ulcers bilat.
Family no. 3					
♀ b. 1955	1975	DVT right leg	Postpartum	No	
♀ b. 1958	1975	DVT right leg	Influenza + pleuritis	No	1983 pregnancy - AT III conc. Delivery - AT III conc. + dextran
Family no. 4					
♀ b. 1916	1941	DVT bilat.	Pregnancy wk. 36	No	1956 pregnancy and delivery no prophylaxis. Leg ulcers bilat.
	1945 later	Recurrent thrombophlebitis	" wk. 10		
	1985	DVT left leg			
♂ b. 1941	1977	DVT left leg	Long journeys by car	Since 1985	
Family no. 5					
♀ b. 1945	1965	DVT left leg	Pregnancy wk. 28	Since 1967	
	1967	" "	" wk. 6		
♂ b. 1965	1982	DVT right leg	Slight trauma, bronchopneumonia	Since 1982	

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
Family no. 6					
♂ b. 1912	1951	DVT left leg	Long journey by car	Since 1961	1954 op. hernia inguinalis right side - no prophylaxis
	1952	" right leg			
	1961	" "			
♂ b. 1937*	1973	DVT right leg	No	Since 1976	1959 op. hernia ing. left side
	1976	+ suspect PE			
♂ b. 1938*	1956	DVT right leg	Ulcus duod. performans Billroth II op	1958-61	1961 ligation of insuff. com-municant veins bilat.
	1958	DVT right leg			
		Thrombophlebitis left leg			
	1959	DVT right leg			
	1960	" "			
	1963	" "			
	1975	" left leg			
	1977	" "			
1978 ligation of insuff. com-municant veins (twice) - dextran prophylaxis					
1980 ligation of insuff. com-municant veins right leg - AT III conc. + dextran					
1981 ligation of insuff. com-municant veins bilat. - AT III conc. + dextran					
1982 ligation of insuff. com-municant veins bilat. - dextran					
1985 ligation of insuff. com-municant veins right leg - dextran					

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♀ b. 1940*	1959	DVT right leg	Pregnancy wk. 26		1974 appendectomy - dextran prophylaxis Leg ulcers bilat.
	1960	" left leg	Postpartum		
	1974	Thrombophlebitis right leg		Since 1974	
	1979	Suspect DVT right leg			
	1984	DVT right leg			
♀ b. 1943*	1967	DVT bilat. + PE	Pregnancy wk. 16		1963 appendectomy-no prophylaxis 1968 pregnancy " " 1973 salp.-oophoect. " " 1976 at delivery (caesarian section) - heparin prophylaxis + dextran 1981 ligation of insuff. communi- cant veins left leg. Leg ulcers bilat.
	1969	" "	Postpartum		
	1976	Thrombophlebitis	Pregnancy first trim.	1977-81	
	1980	DVT left leg			
	1982	Occlusion vertebral artery		Since 1982	
♀ b. 1948*	1968	DVT left leg	Combined contraceptive pills for 3 months	No	1980 heparin prophylaxis - spontaneous abortion - AT III conc. + dextran at evacuatio 1981 heparin prophylaxis during pregnancy, AT III conc. + dextran at delivery Ulcers left leg
	1972	" "	Pregnancy wk. 12		
	1980	" "	" wk. 6		
	1981	" "	" wk. 7		

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
Family no. 7					
♀ b. 1914	1941	DVT left leg	Pregnancy wk. 34		Ulcers right leg
	1946	" " "	" wk. 30		
	1955	" " "			
	later	Recurrent thrombo-phlebitis bilat.			
	1972	Thrombophlebitis	Appendectomy	Since 1977	1972 - no prophylaxis
♀ b. 1939	1960	DVT left leg	Combined contraceptive pills for 2 weeks	Since 1977	1970, 1984 stripping left leg - no prophylaxis 1974 cholecystectomy - no prophylaxis
♀ b. 1949	1976	DVT left leg	Pregnancy wk. 32	Since 1977	Asthma since 7 years of age 1969 pregnancy - no prophylaxis 1984 evacuatio - AT III conc.
Family no. 8					
♀ b. 1936	1972	Suspect PE	Salp.-oophoect.	No	1962 laparoscopy - no prophylaxis 1965, 1968 pregnancy and delivery - no prophylaxis 1971 appendectomy - no prophylaxis 1978 op. goiter - no prophylaxis Varices left leg
♀ b. 1965	1983	DVT left leg	Combined contraceptive pills for 3 months	No	1975 appendectomy - no prophylaxis

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
Additional individual patients					
♂ b. 1927	1955	DVT right leg	No		1976 heparin prophylaxis
	1976	" "	Cholecystectomy		Mother recurrent DVT from young age, no family investigation
	1977	" "			
	1978	" left leg			
	1981	" right leg		Since 1981	
♂ b. 1954	1979	DVT left leg + PE	No	No	No family history of TE, no family investigation
♂ b. 1967	1983	DVT left leg	Fever, coughing	No	No family history of TE, no family investigation
♀ b. 1966*	1983	DVT right leg	Combined contraceptive pills for 3 months	Since 1983	No family history of TE, parents and younger brother normal AT III
					1984 evacuatio - heparin prophylaxis
					1985 abrasion muc.uteri - heparin prophylaxis
♂ b. 1938	1970	DVT right leg	No		No family history of TE
					Parents normal AT III
					1973 stripping right leg - no prophylaxis
					1975 ligation of insuff. communicant veins right leg - no prophylaxis
					Ulcers right leg
♀ b. 1946	1975	DVT right leg (twice)	Pregnancy wk.30 + 2 weeks postpartum	No	1972 pregnancy and delivery - no prophylaxis
					No family history of TE
					Parents normal AT III

Table II. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♀ b.1956*	1979	DVT bilat.	Bronchopneumonia Combined contraceptive pills 3 weeks P&P slightly above at the therapeutical level	Since 1980	1982 evacuatio - AT III conc. No family history of TE No family investigation
	1980	" left leg			
	1982	PE			

Table III. Patients with classic AT III deficiency, but so far without symptoms of TE

Age	Family no.					
	1	2	3	4	5	6
0 - 10				1 F		
11- 20	3 F				1 F	1 F
21- 30	2 F* 1 M			1 M		
31- 40	1 M	1 F				
41- 50	1 F					
51- 60	1 F 1 M					
61-	1 M		1 M			

M = male; F = female

*) Prophylaxis with AT III concentrate + dextran or heparin alone in one caesarian section, one partus normalis, two early abortions and one laparoscopy.

Paper II

Abnormal AT III rarely causes TE. The family presented in this paper appears to be the first case of abnormal AT III discovered in Sweden. In this case the defect seems to be a mutation, there being no family history of thrombus, and AT III being normal in both parents of the propositus, as well as in his older brother and two younger sisters. Otherwise, the inheritance of abnormal AT III is generally reported as being autosomal dominant, which, in our case, seems to be how the defective gene was transmitted to two of the propositus's four children, one boy and one girl. Investigation showed that the abnormality was most similar to the first type of abnormal AT III ever described, namely AT III Budapest, which is characterised by a normal AT III related protein, but reduced thrombin neutralising capacity and anti-Xa activity both in the presence and the absence of heparin. A decreased affinity for heparin was demonstrated with CIE. Investigations using ^{125}I AT III heat-treated concentrate showed a similar half-life (approx. 3.0 days) and fractional catabolic rate in the patient with abnormal AT III as in patients with the classic type of AT III deficiency and as in a control. This indicates that the concentrate was catabolised in the same way in all the subjects we investigated.

Antithrombin III concentrate: Its catabolism in health and in antithrombin III deficiency

Paper III

Pharmacokinetic studies of purified human AT III concentrate are presented in this paper. The concentrate was the original non-heat-treated product from KabiVitrum AB, Sweden, prepared by affinity chromatography on heparin Sepharose (Miller-Andersson et al 1974). The radiolabelling (by one of the authors, L-E N) was performed on albumin-free concentrate, using a iodine monochloride method (McFarlane 1964, Glover et al 1967).

The studies of ^{125}I AT III concentrate, both in the presence and absence of warfarin were carried out on two normals, and on two patients with known congenital AT III deficiency though in a non-thrombotic state. The final amount of AT III injected was 0.3–1.7 mg protein, corresponding to an activity of 0.5–1.3 MBq of ^{125}I (13.5–34 μCi).

A bolus of non-labelled concentrate was given simultaneously with the ^{125}I AT III, in all the cases but one, to determine the gradual decrease of AT III with coagulation assays.

Analysis of AT III before radiolabelling showed the heparin cofactor activity to be only about half of the value assessed by EIA or by clotting assay (the progressive AT III). On CIE, a serum-like pattern appeared with two peaks of about equal height (i.e., different from that of native plasma, in which one high fast-moving peak is followed by two small peaks). However, the second peak of the concentrate moved slower than that of normal serum.

Analysis of the concentrate showed that the radiolabelling procedure did not result in any qualitative changes nor, according to SDS-PAGE (not presented), was there any change in molecular weight after the radiolabelling. On autoradiography (not presented) both peaks on CIE showed the same degree of radioactivity.

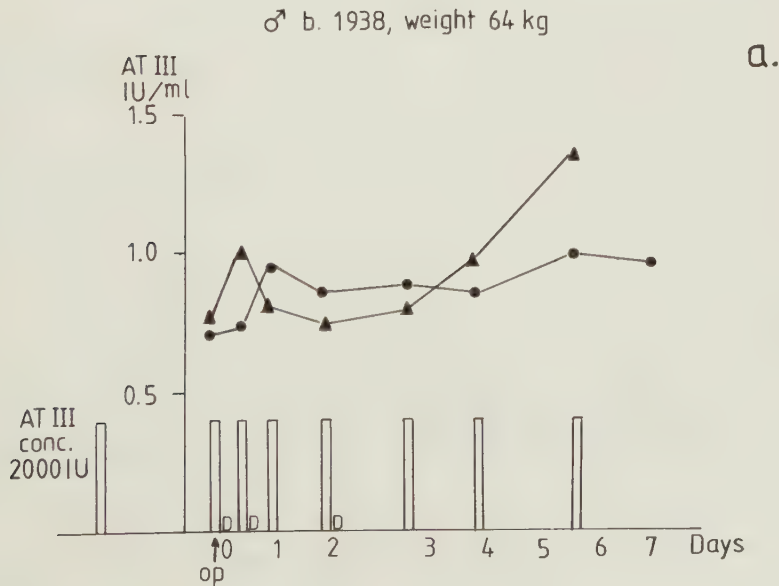
Blood samples were drawn repeatedly for 10–12 days, and urine collected every 24 hours, throughout the observation period. TCA-precipitated protein of the plasma samples was counted in a gamma counter. The plasma values were plotted in a semilog graph of percentage radioactivity against time. The

curves were graphically resolved into exponential functions, according to the routine peeling-off technique. Each curve could be adequately described as the sum of three exponential components. The half-life of each curve was read from its final straight section, but was also calculated from the intercepts of the regression lines of the slowest third phase. From the intercepts and slopes, and using Nosslin formulae (Nosslin 1964), the fractional catabolic rate was also calculated, being expressed as the catabolism per day, and the distribution as the ratio between the total pool and the intravascular pool. As a further check of the values obtained for daily fractional catabolic rate, the radioactivity of the urine samples was compared with that of simultaneous plasma values.

Summing up the results: a) the half-life was 3.4-4.8 days, b) catabolism remained unaffected by warfarin, c) the rate of synthesis in controls was about twice that of those with AT III deficiency, d) there was no difference in half-life, catabolism or distribution between the controls and individuals with congenital AT III deficiency.

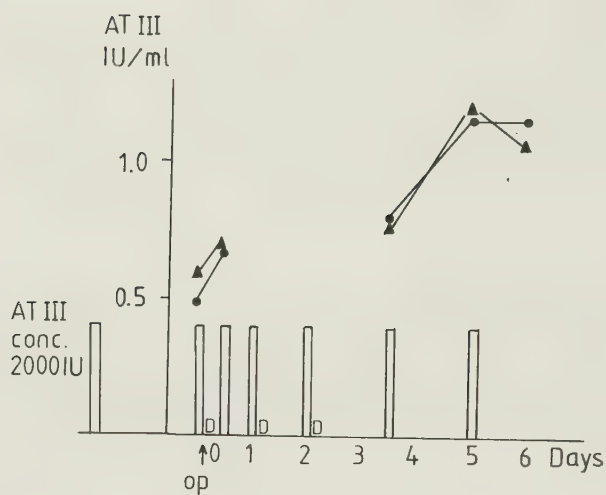
Addendum IIIA

Fig. 5 a-c AT III levels after administration of AT III concentrate (non-heat-treated) at a, and b, ligation of insufficient communicant veins, and c, caesarian section.
 ●—● EIA, ▲—▲ heparin cofactor assay, D = 500 ml Macrodex



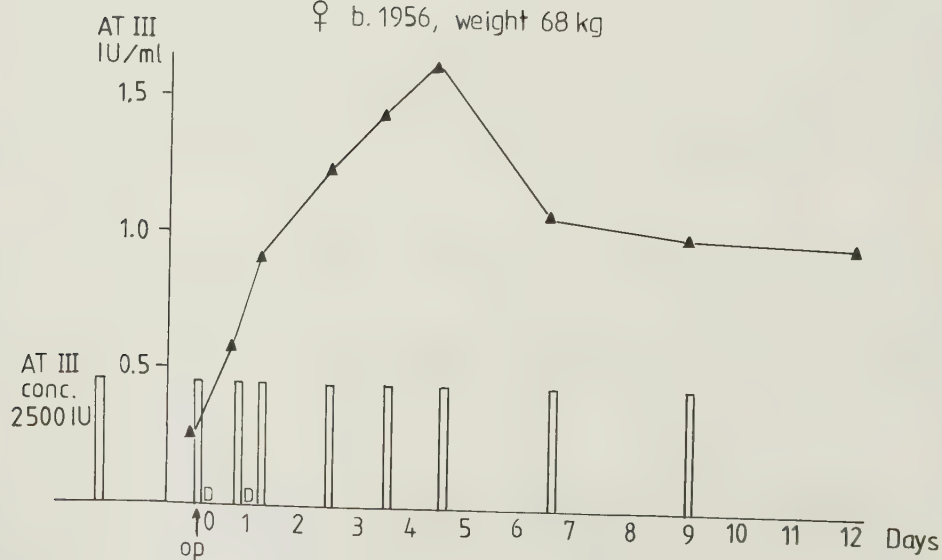
♂ b. 1938, weight 64 kg

b.



♀ b. 1956, weight 68 kg

c.



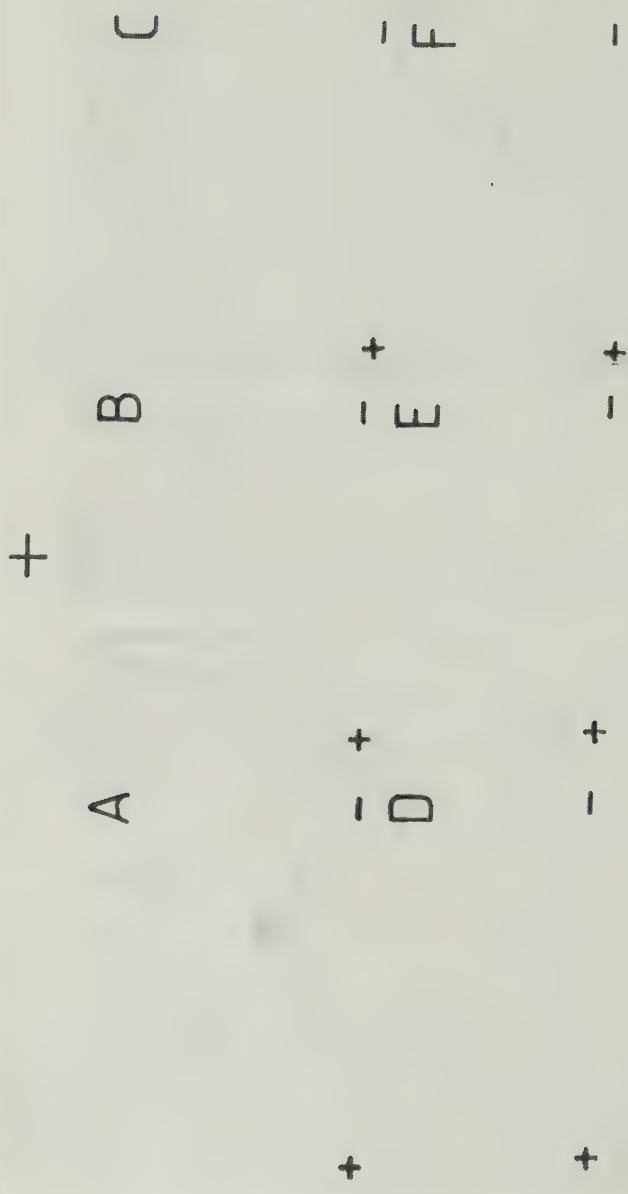
Addendum IIIB

In the same way as described above (Paper III), survival studies were done on heat-treated AT III concentrate. In this product the preparation procedure before the heat-treatment stage had been slightly modified by the manufacturers. In vitro characterisation showed a marked improvement of the heparin cofactor activity (Table IV) with comparable values being obtained of the heparin cofactor, the progressive activity and immunochemically. The pattern of CIE (Fig. 6) was similar before and after heat-treatment. The doses of the AT III concentrate and the radioactivity administered in the in vivo studies are given in Table V. Subject C and D in this study are the same as case I and control II in the study with non-heated AT III concentrate. The results in Table VI shows that the half-life was nearly 25 % shorter (mean 3.0 days; range 2.9-3.2 days) for the heat-treated concentrate than for the original non-heated preparation.

Table IV

The in vitro studies of the heat-treated AT III preparation by various assays

	EIA	Progressive activity clotting assay	Heparin cofactor assay
	IU/ml	IU/ml	IU/ml
AT III concentrate before radiolabelling	0.98	0.98	0.86
¹²⁵ I AT III	0.97	0.96	0.84
AT III concentrate used as bulk dose	1.04	0.96	0.94



Addendum IIIB

Fig. 6 CIE of A, normal serum; B, non-heat-treated AT III; C, heat-treated AT III used as bulk dose;
D, normal plasma; E, heat-treated AT III before radiolabelling; F, 125I AT III.

Table V

Clinical and laboratory data on two patients and one control investigated with heat-treated AT III concentrate

Sub- ject	Method	Baseline AT III IU/ml	Warfarin therapy	Body weight kg	Bulk dose of AT III IU	Tracer dose of ¹²⁵ I AT III		
						MBq	IU	mg
A	EIA	0.41						
	Clotting assay	0.55	Yes	65	2000	1.13	1.9	0.37
	Hep.co factor assay	0.49						
C	EIA	0.43						
	Clotting assay	0.45	Yes	93	2000	1.50	3.3	0.65
	Hep.co factor assay	0.46						
D	EIA	1.00						
	Clotting assay	1.00	No	73	1500	1.36	3.0	0.59
	Hep.co factor assay	1.18						

Table VI

Calculated parameters based on the studies of heat-treated ¹²⁵I AT III concentrate

	Plasma pool* g	T/P	FCR ₋₁ days	U/P	Final T _{1/2} days	Rate of synthesis* g/day
A	0.251	2.5	0.71	0.47	3.0	0.178
C	0.332	2.2	0.59	0.55	3.2	0.196
D	0.619	2.7	0.64	0.54	2.9	0.396

*) Assuming a plasma volume of 0.04 l/kg body weight

FCR = fractional catabolic rate

T/P = the ratio between total and intravascular pool

U/P = the radioactivity in the urine samples compared with plasma values

T_{1/2} = half-life

A family with thromboembolic disease associated with deficient fibrinolytic activity in vessel wall

Paper IV

Altogether 17 members of a large family with a high incidence of DVT were investigated. Of the 13 patients who had had DVT, 11 had defective vessel wall fibrinolysis when analysed after stimulation by venous occlusion for 20 minutes. Fibrinolytic activity was assayed on fibrin plates, the examination being performed on two consecutive days. In the 11 patients with DVT and decreased fibrinolytic activity, the abnormality could be demonstrated only on the second day of examination in 9 patients; thus only two showed low fibrinolytic activity on both days of investigation. This suggests that the venous occlusion test should be performed on two consecutive days.

The fibrinolytic activity of biopsy specimens, using the fibrin slide autography technique, showed normal values in all the patients studied.

Other examined components of the coagulation and fibrinolytic systems known to predispose to the development of TE were normal.

This is one of the first described families in which decreased fibrinolytic activity of the vessel wall was found in association with DVT.

Paper V

Two members of the family with constantly defective vessel wall fibrinolysis presented in Paper IV were re-investigated using methods recently developed. Firstly, biopsy specimens were examined using the histochemical autography technique with monospecific antibodies against melanoma activator or UK included in the fibrin film. When anti-melanoma activator was added, the fibrinolytic activity of the biopsy was totally quenched but the activity remained unaffected when anti-UK or control IgG was added. These findings suggest that the activity in the vessel wall was immunochemically identical with the t-PA derived from the melanoma activator.

Secondly, the fibrinolytic activity after venous occlusion was assayed by IRMA using the above mentioned antibodies. Also the new functional method using the chromogenic substrate S-2251 was applied. There was a marked discrepancy in results between the two assays, values being normal according to IRMA but abnormally low according to the functional assay. Further investigations revealed a high activity of a t-PA inhibitor. This was especially evident in the younger patient whose pre-occlusion plasma, on the second day of examination, had an inhibitor value 50 times higher than in the reference population. Using SDS-PAGE followed by zymographic analysis, fibrinolytically active bands were demonstrated with similar molecular weight both in patients and controls (approx. 100,000), and probably consisting of complexes between the t-PA and its inhibitor. Also using SDS-PAGE and zymographic analysis, it seemed that the inhibitor formed complex not only with melanoma activator, but also with UK (later demonstrated, Fig. 7) and the pattern was as a principle the same for both patients and controls, but the inhibitor capacity to bind to activators was considerably higher in the patients.

Addendum V

Table VII. Brief clinical data on the patients presented in Paper V.

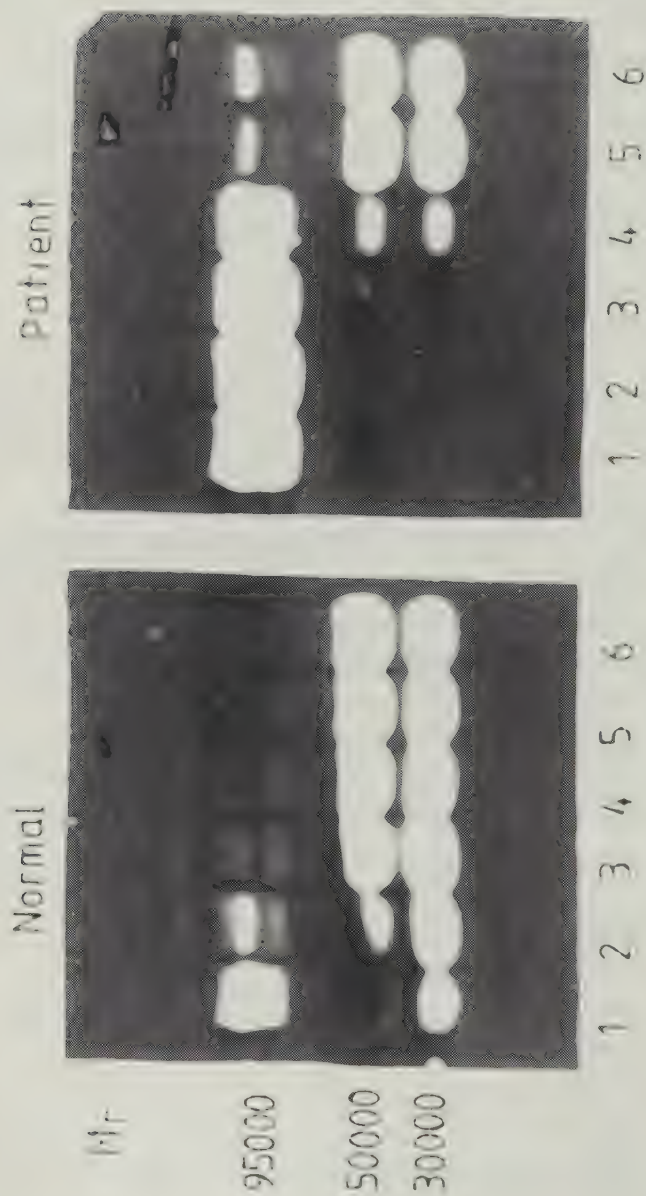


Fig. 7 SDS-PAGE followed by zymographic analysis of normal and patient pre-occlusion plasma in various dilutions 1-6 (1:10 - 1:500) mixed with 1 Ploug unit urokinase (Serenio, Italy).
 M_r 30,000 and 50,000).

Addendum V

Table VII

Brief clinical data on the patients presented in Paper V.

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♀ b.1907 III:2	1926	DVT	Postpartum		1959 gynaecological op. - Coumarin prophylaxis
	1928	"	"		1962 cholecystectomy - Coumarin prophylaxis
	1935	"	Pregnancy		
	1944	"	" + postpartum		
	1961	"	Long bed rest		
	1965	Thrombophlebitis left arm	Salp. oophoect.	No	
♂ b.1916 III:7	1936	DVT	No		
	later	Recurrent DVT and thrombophlebitis		Since 1973	
♂ b.1933 IV:1	Since early 50s	Recurrent DVT and thrombophlebitis	No		1974 cholecystectomy - no prophylaxis
	1981	DVT right leg		Since 1981	
♂ b.1928 IV:3	1939	DVT bilat.	Bronchopneumonia		1972 stripping right leg 1975 ligation of insuff. communicant veins bilat. 1978 ligation of insuff. communicant veins right leg
	1940s- 50s	Recurrent thrombo- phlebitis legs and arms		Since 1961	
♂ b.1929 IV:7	1972	DVT left leg	No	1972-75	Ulcers left leg
	1975	"			
	1976	"			
	1978	"		Since 1976	

Table VII. Continued

Patient	Year	Diagnosis	Known trigger	Cont. oral anticoagulants	Remarks
♀ b.1939 IV:9	1960s	Recurrent thrombo- phlebitis legs and arms	No		1960, 1962 pregnancy and delivery - no prophylaxis
	1974	DVT left leg			
	1975	" " " + PE		No	1983 abrasion muc. uteri - no prophylaxis
♀ b.1941 IV:10	1975	Thrombophlebitis	Drawing blood sample	No	Three pregnancies and deliveries - no prophylaxis
♀ b.1930 IV:13	1953	DVT bilat.	Postpartum		Six pregnancies and deliveries between 1957-66 - no prophylaxis
	1967	" left leg	Cholecystectomy	No	Leg ulcerations bilat.
	1975	" " " + PE			Three pregnancies and deliveries without TE
♀ b.1945 IV:18	1975	DVT left leg	No		1980 prophylaxis with dextran during delivery
	1976	" " "			
	1980	Thrombosis sinus sagittalis	3 weeks postpartum	Since 1980	
♂ b.1943 IV:22	1975	DVT left leg	No	1975-80	
	1980	" right leg		Since 1980	
♂ b.1954 IV:25	1984	PE	No	No	
♂ b.1959 IV:26	1974	DVT left leg + PE	No	Since 1974	
♀ b.1953 V:9	1973	Thrombophlebitis	Pregnancy	No	Two other pregnancies and deliveries without TE - no pro- phylaxis
	1981	"	Postpartum		

GENERAL DISCUSSION

The pathogenesis of DVT is complex, being in most cases a result of several accidental and cumulative factors without the precise mechanisms being known. In some cases, however, and particularly in patients who have had their first DVT at an early age, congenital predisposing irregularities in the blood or vessel wall may be found. The occurrence of DVT may be familial, and investigations of such families have revealed various individual predisposing defects in the coagulation and fibrinolytic systems to be common to family members. While one of the best-defined abnormalities is AT III deficiency, the most frequent seems to be defective vessel wall fibrinolysis. Although other congenital abnormalities in the circulating blood, such as plasminogen or protein C deficiency are also recognised in conjunction with TE, their occurrence seems to be more rare.

Antithrombin III

Since 1965, when Egeberg first drew attention to decreased AT III as a familial thrombogenic factor, several such families have been described. In Egeberg's original paper, AT III was measured functionally as the thrombin inhibiting activity in the presence or absence of heparin. A few years later an immunochemical method for measuring AT III became available (Fagerhol & Abildgaard 1970), which was easier to perform and thus became the method often preferred for screening purposes until the new improved functional assays using chromogenic substrates were introduced in the mid 1970s (Ødegård et al 1975). At Malmö, electroimmunoassay was used routinely from the beginning of 1970 (Hedner & Nilsson 1973) (Paper I) and the amidolytic heparin cofactor assay from 1978 (Papers II & III).

By means of the different assays, various types of AT III have been diagnosed which fall into two main categories: type 1 or the so-called classic type (Paper I and Addendum I), with a parallel decrease in both functional and immunochemical assays and type 2 or abnormal types with a normal protein concentration but decreased activity (activities). Several abnormal types have now been described, and are differentiated with regard to the various functions of the AT III molecule (Sas 1984).

The classic type seems to occur considerably more often than the abnormal types, and this could be confirmed among our patients. At the Department for Coagulation Disorders in Malmö, eight families with the classic type have been

diagnosed and a further seven cases of permanently low AT III have been encountered where either no heredity could be found or where family investigation was infeasible (Paper I and Addendum I). So far only one family with abnormal AT III has been diagnosed (Paper II).

Both the classic AT III deficiency and the abnormal variants seem to be inherited in an autosomal dominant manner, and most of the patients reported have been heterozygotes. In AT III Budapest, the abnormality has been found to vary in degree from one affected family member to another (Sas et al 1980), which is probably to be explained by consanguinity with a homozygote (the propositus) and heterozygotes. The first Swedish patient with abnormal AT III (Paper II), was a young male with a thrombotic incident but no family history of thrombosis. This patient was obviously a heterozygote, judging by the slightly diminished values in the various assays. Moreover, a mutation seems to have appeared in the propositus and to have been transmitted to two of the propositus's four children. Spontaneous mutation has been reported earlier in one family, in which study even the AT III locus in the chromosome was identified (Winter et al 1982).

The first family reported with abnormal AT III, AT III Budapest (Sas et al 1974), seems to have a general molecular defect, with decreased inhibiting capacity both for thrombin and Xa, as well as reduced heparin binding function. In all probability, our patient (Paper II) represents such a variant with a similarly generalised defect, and unlike other familial variants such as AT III Basel, Paris and Toyama, in which the progressive thrombin inhibiting activity was normal but the heparin-AT III reaction was defective. Moreover, unlike AT III Aalborg, in our patient the Xa inhibiting activity was decreased (Table I, p. 16).

Although thrombosis is frequent in patients with the classic type of AT III deficiency, and occurs in most families with abnormal AT III, congenital AT III deficiency is a rare disorder among those with TE. Thus, of about 4000 thrombosis patients referred to the Department for Coagulation Disorders in Malmö since 1970, altogether only 37 were found to have the classic type and one the abnormal defect. This represents about 1 % of all thrombosis patients, which is similar to figures reported by others (Rosenberg 1975, Lechner et al 1977), though our figure is based on patients referred from various other hospitals for investigation because of thrombosis, which means that the material is somewhat selected. Although the prevalence is unknown, Abildgaard (1979) found five women with AT III deficiency in a large study of 25,000.

All but one of our patients had venous thrombosis, a finding confirming earlier observations that venous thrombosis is considerably more common than arterial (Winter & Douglas 1983).

There has been much discussion as to the optimal treatment in cases of acute DVT or PE in patients with AT III deficiency. The advisability of heparin treatment has been questioned (Filip et al 1976, Marciniak & Gockerman 1977), since heparin was said to cause yet further decrease of AT III. The administration of heparin to patients with congenital AT III deficiency (usually 40–60 % of normal) will produce a further decrease of AT III to 20–30% (Hellgren et al 1982). Nonetheless, we found, when giving heparin by continuous infusion, the APTT to be sufficiently prolonged (Fig. 8); these findings have received support from an experimental study by Sie & Boneu (1983), who showed that, with AT III concentrations of 20–30 %, full anticoagulant effect of heparin was obtained as measured by APTT. However, although rarely used in Sweden, thrombolytic therapy may be the best choice in acute TE, as it may reduce the risk of post-thrombotic syndrome.

To prevent the recurrence of DVT, we generally recommend life-long prophylaxis with oral anticoagulants after the second thrombotic incident, but the indications should be assessed from case to case, and such factors as the site of a thrombus and the degree of post-thrombotic syndrome must be taken into account. This may seem to be a rather conservative approach as compared with those recommended by others; some suggest continuous prophylaxis in AT III deficient patients who have suffered only one TE event, or even in those who have attained the age of 20 without any TE episodes at all (Winter & Douglas 1983). At present, it has not been established whether, and under what circumstances, anticoagulant prophylaxis is indicated in a subject who has not had any thrombotic symptoms. Among patients seen at Malmö, not less than 17 out of 54 with the classic AT III deficiency has not so far had any symptoms of TE; of these 17 patients, 11 were more than 20 years of age. Medication with combined (oestrogen and progesterone) contraceptive pills is considered contraindicated, as the oestrogen component may cause a further decrease of AT III (Conard et al 1980) as well as reduced vessel wall fibrinolysis (Kjeldgaard & Larsson 1983).

In most patients with a congenital AT III deficiency the trigger for TE can be traced. As seen in the Addendum I to Paper I, the frequency of DVT is high in pregnancy and often appears in the first trimester; not included in the Addendum is one woman (belonging to the family presented in Paper I) who died of PE following delivery. The choice of prophylaxis during pregnancy and delivery is

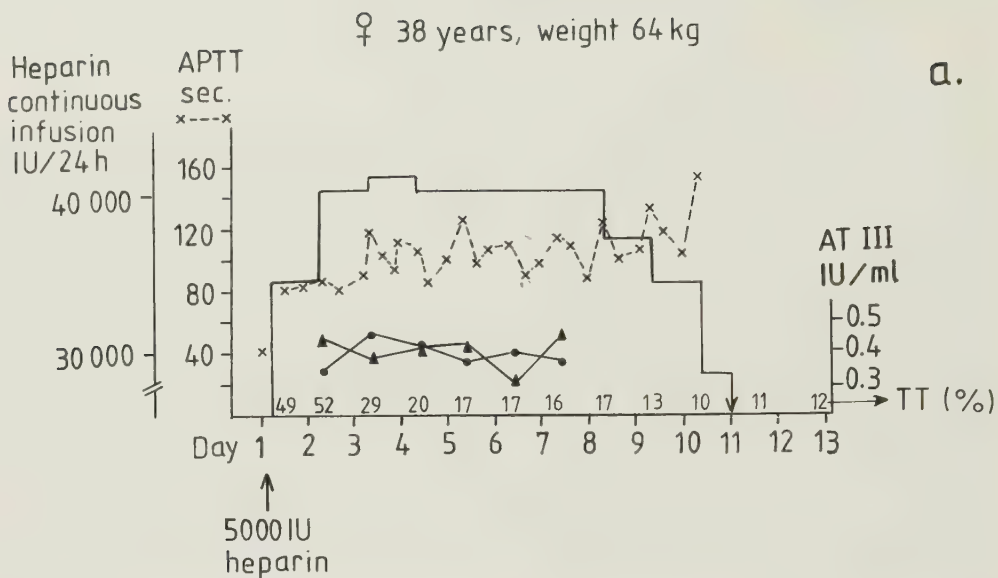


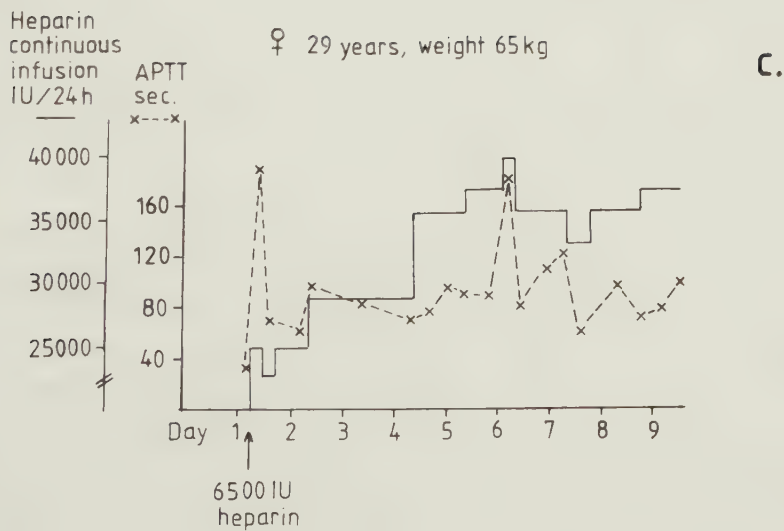
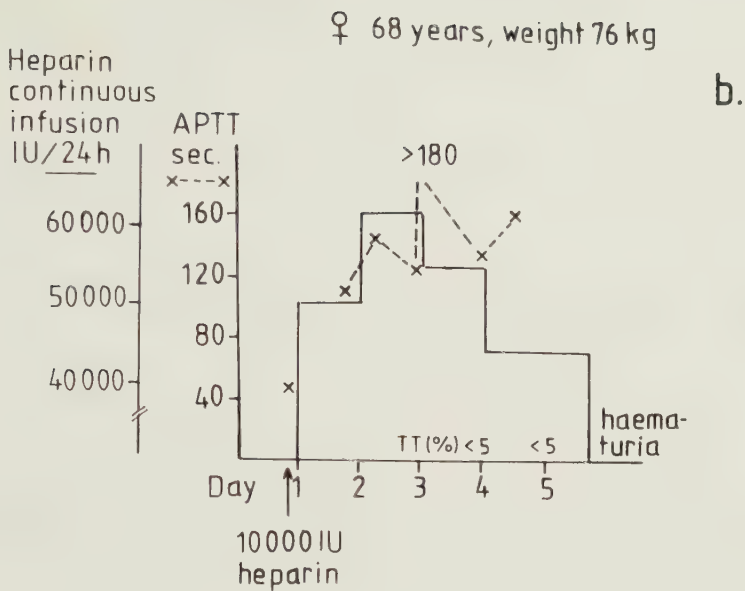
Fig. 8 a-c The APTT during heparin treatment in three patients (a-c) with congenital AT III deficiency in acute thrombosis. In a, and b, Waran medication was started on the first day of heparin infusion.

x---x APTT

●---● EIA

▲---▲ heparin cofactor assay

TT = Thrombotest



a crucial one, and constitutes a special problem since oral anticoagulants are contraindicated, at least during early pregnancy, and the advisability of heparin treatment has been questioned owing to the risk of side effect. Despite the risk of developing osteoporosis (Griffith et al 1965, De Swiet et al 1983, Letsky & De Swiet 1984), however, heparin prophylaxis seems to reduce the frequency of TE, at least according to a report on a small group of patients by Hellgren et al (1982).

The rational treatment in congenital AT III deficiency ought to be normalisation of AT III by plasma or an AT III concentrate. AT III concentrate may be used, for example, as prophylaxis during pregnancy (Tengborn & Bengtsson 1985), or at deliveries and operations (Mannucci et al 1982) (Addendum I). To render treatment with an AT III concentrate preferable to giving plasma, the existence of an efficient and safe concentrate has first to be established. The manufacturer of the original non-heat-treated Swedish AT III concentrate (Kabi-Vitrum AB) later introduced heat treatment of AT III in solution. It has recently been shown that heat treatment is effective against HTLV III, the agent of the dreaded AIDS (acquired immune deficiency syndrome) (Spire et al 1985). The in vitro studies of the original non-heated product (Paper III) showed a substantially decreased heparin cofactor activity, as compared with the progressive antithrombin activity and with the AT III related protein, suggesting an altered AT III molecule with a denatured heparin binding site but with a thrombin-inhibiting site that remained largely intact. These findings were later confirmed by others (Barrowcliffe et al 1983). Later on, by a slight modification of the early stages of the preparation procedure, the heat-treated product was much improved with regard to its heparin-binding capacity, an improvement that remained unaffected by the subsequent heat-treatment phase of the preparation procedure (Addendum IIIB).

In vivo studies of the original concentrate (Paper III) showed that, in steady state conditions, the rate of AT III synthesis in patients with the classic AT III deficiency was about half of that in controls. The fractional catabolic rate was roughly the same in patients and controls, as was the half-life (3.4-3.8 and 3.8-4.8 days, respectively) - i.e., similar to the findings of Scully et al (1981), but somewhat longer than that reported by Collen et al (1977) who found a mean half-life of 2.8 days. The half-life of heat-treated concentrate (Addendum IIIB) was almost 25 % shorter (2.9-3.2 days) than that of the non-heated preparation, although longer half-life might have been expected since the in vitro assay results seemed to show a well-preserved AT III molecule with normal heparin-binding properties.

The half-life of the AT III concentrate was similar in the patient with the abnormal variant (Paper II) and in patients with the classic AT III deficiency, indicating that the AT III concentrate was similarly catabolised in vivo in all our patients and in controls.

Conflicting results have been obtained regarding the behaviour of AT III in patients on oral anticoagulant medication, and several workers have reported increase in AT III concentrations and activity (review, Manotti et al 1982). However, in our studies there was no difference in half-life, whether warfarin was present or not, either in controls or patients with the classic deficiency (Paper III).

There were no clinical signs of hepatitis in any of the patients treated either with the original AT III concentrate or the heat-treated product, and a six month follow-up of some of the patients failed to detect any increase of their serum ALAT (alanine-aminotransferase) content.

Defective vessel wall fibrinolysis

A more frequent defect among patients with thrombotic disease appears to be defective fibrinolysis. Judging from our experience at the Department for Coagulation Disorders in Malmö, thromboses in veins and arteries occur fairly often (38-72 %), in conjunction with an abnormally low vessel wall fibrinolysis (Isacson & Nilsson 1972, Sundqvist et al 1981, Tengborn et al 1981).

Defective fibrinolysis in conjunction with thrombosis may also be a familial phenomenon. We described one of the first families with such an abnormality (Paper IV), which was inherited in an autosomal dominant manner. Andersen (1976), Jørgensen et al (1982) and Stead et al (1983) described one family each with similar findings. Since the spontaneous fibrinolytic activity in circulating blood is low, some type of stimulation is often used to enable it to be measured at all. In our family, stimulation by venous occlusion or DDAVP resulted in a lower fibrinolytic activity on fibrin plates than was seen in a reference population. Biopsy specimens of the vessel wall showed normal fibrinolysis, according to a fibrinolytic autography technique. Such a pattern might be due either to impaired release of fibrinolytic activity into the blood stream, to release of an abnormal activator activity, or to increased inactivation of fibrinolytic activity owing to an excess of inhibitors. It should be mentioned that, after venous occlusion, fibrinolytic activity was normal in several of our family members on

the first day of examination but abnormally low on the second day. Two members of the family described in Paper IV were later investigated with regard to t-PA antigen, t-PA activity and inhibitors of t-PA (Paper V). Interestingly, after venous occlusion these patients released normal levels of t-PA related substance, as measured in an IRMA, but markedly decreased t-PA according to an amidolytic method, a discrepancy indicating that the defect might either be due to the release of an abnormal activator with reduced capacity to bind to fibrin, or to the activator being inactivated by an inhibitor. Further investigations showed an increased plasma inhibitor activity, particularly in samples drawn before venous occlusion on the second day of the examination. This was more pronounced in the younger of the two patients, perhaps because he was more severely affected than the other one. The abnormality displayed in our family seems to be a rather common one (Haglund et al 1984, Nilsson et al 1985b).

The defect demonstrated in our family (Paper V) may be caused by an increased synthesis and/or release of the fast-acting inhibitor of t-PA although it can not be excluded that the inhibitor is abnormal. A similar pattern with markedly increased t-PA inhibitors has been described in severely ill patients with varying diagnoses (Juhan-Vague et al 1984, Colucci et al 1985).

In the future, when t-PA becomes available for treatment, it may be of clinical importance that, for instance, the level of t-PA inhibitor is known in order to be able to assess optimal dosages. Currently, the routine treatment recommended in acute thrombosis is heparin and oral anticoagulants, or in patients with no contraindications for thrombolytics, either SK or UK. Since shut-down of fibrinolysis (review, Bergqvist 1983), and an increase in t-PA inhibitor occur post-operatively (Juhan-Vague et al 1984, Kluft et al 1984) in cases of permanently reduced fibrinolysis, it seems indicated to be liberal with thrombo-prophylaxis (heparin or dextran), even in minor surgery and young patients.

Little has been reported about defective vessel wall fibrinolysis and DVT during pregnancy, though it is known that fibrinolysis gradually decreases while the t-PA inhibitor successively increases (Åstedt et al 1984, Wiman et al 1984). Although our material is small, it is striking that in our family with the defective fibrinolysis only a few cases had DVT during pregnancy as compared with the AT III deficient patients (Addendum I and V). Of course, the material needs to be extended before any firm conclusions can be drawn, and long term follow-up is required before the question of prophylaxis during pregnancy can

be settled.

The notorious complexity of the pathogenesis of DVT, which even today is still best summarised in Virchow's triad, constitutes a formidable obstacle to adequate prophylaxis and treatment. As the different defects and their variants become successively clearer to us, which is perhaps most easily achieved through familial studies, more light will be shed on our search for specific and effective means of dealing with thrombosis.

CONCLUSIONS

- AT III deficiency is a rare cause of thrombosis. At the Department for Coagulation Disorders in Malmö, about 54 patients (8 families) have been diagnosed as AT III deficient out of about 4000 patients with thrombotic diseases. The defect is inherited in an autosomal dominant manner; mutations may occur. All of the 54 patients were heterozygotes with AT III values 40–60 % of normal. These patients had the so-called classic type with a normally functioning protein showing partially decreased values both in immunochemical and various functional methods. Since the incidence of venous thrombosis is high (37 patients, 69 % in our material), family investigations are important especially with regard to the choice of thrombo-prophylaxis. The first thrombosis often occurs before 25–30 years of age, and in conditions known to predispose to thrombosis.
- Abnormal variants of AT III are also associated with thrombosis, but such defects seem to be extremely rare. At Malmö only one patient has so far been diagnosed. The abnormality appeared to be a mutation, the inheritance of which was autosomal dominant. The AT III related antigen was normal but the thrombin inhibiting capacity as well as the anti-Xa activity, both in the presence and absence of heparin, were partially decreased. Moreover, the pattern of CIE with heparin included in the gel in the first dimension was abnormal.
- In AT III deficiency, normalisation of AT III is the most rational treatment. The original Swedish AT III concentrate had a relatively low heparin cofactor activity, that was subsequently increased. In addition, it was heat treated thereby diminishing the risk of transmitting AIDS. The mean half-life of the original concentrate was 3.9 days; range 3.4–4.8 days in steady state; there was no substantial difference in patients or in controls. The rate of synthesis in patients was about half that of controls. Medication with warfarin did not affect the half-life.

In the abnormal AT III variant the AT III concentrate was catabolised at the same rate in controls as in patients with the classic AT III deficiency.

- In acute DVT or PE in patients with AT III deficiency or abnormal AT III, routine treatment with heparin, preferably in continuous infusion, as well as oral anticoagulants, is sufficient; addition of AT III concentrate is not

required, although the heparin will cause some further reduction of the AT III level. In major surgery and deliveries, specific thrombo-prophylaxis with AT III concentrate is indicated. After two or more attacks of TE, life-long prophylaxis with oral anticoagulants is recommended but with the proviso that each case be judged individually. Combined (oestrogen-progesterone) contraceptive pills are contraindicated.

- Deficient vessel wall fibrinolysis, as a predisposing factor with regard to TE, may be a familial occurrence. One type of such a defect seems to consist of a normal release of t-PA related substance, but a decreased activity owing to inactivation of its rapid inhibitor.

REFERENCES

- Aberg M. On effect of dextran on lysisability and structure of ex vivo thrombi, platelet function and factor VIII. Malmö, Sweden 1978 (Thesis).
- Aberg M & Rausing A. The effect of dextran 70 on the structure of ex vivo thrombi. *Thromb Res* 1978; 12:1113.
- Abildgaard U. Highly purified antithrombin III with heparin cofactor activity prepared by disc electrophoresis. *Scand J Clin Lab Invest* 1968; 21:89.
- Abildgaard U. Binding of thrombin to antithrombin III. *Scand J Clin Lab Invest* 1969; 24:23.
- Abildgaard U. A review of antithrombin III. In: *The Physiological Inhibitors of Coagulation and Fibrinolysis* (ed. D Collen, B Wiman & M Verstraete). Elsevier/North-Holland Biomedical Press 1979, p. 19.
- Abildgaard U. Antithrombin and related inhibitors of coagulation. In: *Recent Advances in Blood Coagulation* (ed. L. Poller). Churchill Livingstone, London 1981, vol. 3, p. 151.
- Abildgaard U, Gravem K & Godal HC. Assay of progressive antithrombin in plasma. *Thromb Diathes Haemorrh* 1970; 224:24.
- Abildgaard U, Lie M & Ødegård OR. Antithrombin (heparin cofactor) assay with "new" chromogenic substrates (S-2238 and Chromozym TH). *Thromb Res* 1977; 11:549.
- Alexandre P, Larcen A & Briquel ME. Recurring thrombo-embolic accidents caused by family-related deficiency of the fibrinolysis system. *Blut* 1980; 41:437.
- Ambrus JL, Ambrus CM, Lillie MA et al. Effect of various estrogen treatment schedules on antithrombin III levels. *Res Comm Chem Pathol Pharmacol* 1976; 14:543.
- Ambruso DR, Leonard BD, Bies RD et al. Antithrombin III deficiency: Decreased synthesis of a biochemically normal molecule. *Blood* 1982; 60:78.
- Andersen P. Hyperlipidaemia and reduced fibrinolytic activity associated with thromboembolic complications in a family. *Acta Med Scand* 1976; 200:289.
- Andersson G, Fagrell B, Holmgren K et al. Antithrombin III in patients with deep vein thrombosis during heparin treatment (subcutaneous and intravenous) and during and after treatment with oral coumarins. *Thromb Res* 1984; 34:333.
- Andersson L-O, Barrowcliffe TW, Holmer E et al. Anticoagulant properties of heparin fractionated by affinity chromatography on matrix-bound antithrombin III and by gel filtration. *Thromb Res* 1976; 9:575.
- Aoki N & von Kaulla KN. The activation of vascular plasminogen activator from human cadavers and a description of some of its properties. *Am J Clin Pathol* 1971; 55:171.
- Aoki N, Moroi M, Sakata Y et al. α_2 -plasmin inhibitor: primary natural inhibitor of fibrinolysis. In: *Progress in Fibrinolysis* (ed. JF Davidson, IM Nilsson & B Åstedt). Churchill Livingstone, London 1981, vol V, p.144.
- Aoki N, Moroi M, Sakata Y et al. Abnormal plasminogen. A hereditary molecular abnormality found in a patient with recurrent thrombosis. *J Clin Invest* 1978; 61:1186.
- Arnesen H, Høiseth A & Ly B. Streptokinase or heparin in the treatment of deep vein thrombosis. *Acta Med Scand* 1982; 211:65.

- Astedt B & Holmberg L. Immunological identity of urokinase and ovarian carcinoma plasminogen activator released in tissue culture. *Nature* 1976; 261:595.
- Astedt B, Holmberg L, Lecander I & Thorell J. Radioimmunoassay of urokinase for quantification of plasminogen activators released in ovarian tumour cultures. *Eur J Cancer* 1981; 17:239.
- Astedt B, Holmberg L & Nilsson IM. An inhibitor of t-PA in pregnant plasma. *Haemostasis* 1984; 14:101 (abstract).
- Astrup T & Permin PM. Fibrinolysis in the animal organism. *Nature* 1947; 169: 681.
- Astrup T & Stage A. Isolation of a soluble fibrinolytic activator from animal tissue. *Nature* 1952; 170:929.
- Bachmann F & Kruithof EKO. Tissue plasminogen activator: Chemical and physiological aspects. *Sem Thromb Hemostas* 1984; 10:6.
- Ball AP & Day ED. Immunological identification of two urokinases. *Thromb Diathes Haemorrh* 1970; 24:475.
- Barrowcliffe TW, Eggleton CA & Mahmoud AM. Studies of the heterogeneity of antithrombin III concentrates. *Br J Haematol* 1983; 55:37.
- Bauer KA, Ashenhurst JB, Chediak J & Rosenberg RD. Antithrombin "Chicago": A functionally abnormal molecule with increased heparin affinity causing familial thrombophilia. *Blood* 1983a; 62:1242.
- Bauer KA, Teitel JM & Rosenberg RD. Assays for the quantitation of anti-thrombin III thrombin-antithrombin complex and prothrombin activation fragments. In: *Disorders of Thrombin Formation* (ed. RW Colman). Churchill Livingstone 1983b; p. 142.
- Baumgartner HR. The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi. *Microvasc Res* 1973; 5:167.
- Begent N & Born GVR. Growth rate in vivo of platelet thrombi, produced by iontophoresis of ADP, as a function of mean blood flow velocity. *Nature* 1970; 227:926.
- Bergqvist D. Postoperative Thromboembolism. Frequency, etiology, prophylaxis. Springer-Verlag, Berlin, Heidelberg, New York 1983.
- Bergsdorf N, Nilsson T & Wallén P. An enzyme linked immunosorbent assay for determination of tissue plasminogen activator applied to patients with thromboembolic disease. *Thromb Haemostas* 1983; 50:740.
- Bertina RM, Broekmans AW, Krommenhoek-van Es C & van Wijngaarden A. The use of a functional and immunologic assay for plasma protein C in the study of the heterogeneity of congenital protein C deficiency. *Thromb Haemostas* 1984; 51:1.
- Bjarke B, Herin P & Blombäck M. Neonatal aortic thrombosis. A possible clinical manifestation of congenital antithrombin III deficiency. *Acta Paediat Scand* 1974; 63:297.
- Björk I, Jackson CM, Jörnvall H et al. The active site of antithrombin. Release of the same proteolytically cleaved form of the inhibitor from complexes with factor IX_a, faktor X_a, and thrombin. *J Biol Chem* 1982; 257:2406.
- Blombäck B, Blombäck M & Olsson P. Studies on thrombin inactivation in normal plasma, serum and plasma fractions, and its relation to heparin. *Thromb Diathes Haemorrh* 1963; 9:368.
- Boey ML, Colaco CB, Gharavi AE et al. Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anti-coagulant. *Br Med J* 1983; 287:1021.

- Booyse FM, Scheinbuks J, Radek J et al. Immunological identification and comparison of plasminogen activator forms in cultured normal human endothelial cells and smooth muscle cells. *Thromb Res* 1981; 24:495.
- Born GVR. Fluid-mechanical and biochemical interactions in haemostasis. *Br Med Bull* 1977; 33:193.
- Brakman P, Mohler Jr ER & Astrup T. A group of patients with impaired plasma fibrinolytic system and selective inhibition of tissue activator-induced fibrinolysis. *Scand J Haematol* 1966; 3:389.
- Brandt P, Jespersen J & Gregersen G. Postpartum haemolytic-uraemic syndrome successfully treated with antithrombin III. *Br Med J* 1980; 280:449.
- Brinkhous KM, Smith HP, Warner ED & Seegers WH. An unidentified substance which acts in conjunction with heparin to prevent the conversion of prothrombin into thrombin. *Am J Physiol* 1939; 125:683.
- Broekmans AW, Veltkamp JJ & Bertina RM. Congenital protein C deficiency and venous thromboembolism. A study of three Dutch families. *N Engl J Med* 1983; 309:340.
- Broekman MJ, Handin RI & Cohen P. Distribution of fibrinogen, and platelet factors_{II} and XIII in subcellular fractions of human platelets. *Br J Haematol* 1975; 31:51.
- Brogden RN, Speight TM & Avery GS. Streptokinase: A review of its clinical pharmacology, mechanism of action and therapeutic uses. *Drugs* 1973; 5:357.
- Broze Jr GJ & Majerus PW. Purification and properties of human coagulation factor VIII. *J Biol Chem* 1980; 255:1242.
- Büller HR, Weenink AH, Treffers PE et al. Severe antithrombin III deficiency in a patient with pre-eclampsia. Observations on the effect of human AT III concentrate transfusion. *Scand J Haematol* 1980; 25:81.
- Busch PC. Sulfated glycosaminoglycans and vascular endothelial cells. In: *Biology of Endothelial Cells* (ed. EA Jaffe). Martinus Nijhoff Publishers, The Hague 1984, chapter 17, p. 178.
- Busch C & Owen WG. Identification in vitro of an endothelial cell surface co-factor for antithrombin III. Parallel studies with isolated perfused rat hearts and microcarrier cultures of bovine endothelium. *J Clin Invest* 1982; 69:726.
- Camiolo SM, Markus G, Evers JL et al. Plasminogen activator content of neoplastic and benign human prostate tissues; fibrin augmentation of an activator activity. *Int J Cancer* 1981; 27:191.
- Canfield WM & Kisiel W. Evidence of normal functional levels of activated protein C inhibitor in combined factor V/VIII deficiency disease. *J Clin Invest* 1982; 70:1260.
- Carvalho A & Ellman L. Hereditary antithrombin III deficiency. Effect of antithrombin III deficiency on platelet function. *Am J Med* 1976; 61:179.
- Cash JD. Neurohumoral pathways associated with the release of plasminogen activator in man. In: *Progress in Chemical Fibrinolysis and Thrombolysis* (ed. JF Davidson, M Samama & PC Desnoyers). Raven Press, New York 1975, vol. 1, p. 97.
- Castellino FJ. Biochemistry of human plasminogen. *Sem Thromb Hemostas* 1984; 10:18.
- Chan V & Chan TK. Antithrombin III in fresh and cultured human endothelial cells: A natural anticoagulant from the vascular endothelium. *Thromb Res* 1979; 15:209.

- Chandra T, Stackhouse R, Kidd VJ & Woo SLC. Isolation and sequence characterization of a cDNA clone of human antithrombin III. *Proc Natl Acad Sci* 1983; 80:1845.
- Chmielewska J, Rånby M & Wiman B. Evidence for a rapid inhibitor of tissue plasminogen activator in plasma. *Thromb Res* 1983; 31:427.
- Clarke RL, Orandi A & Cliffton EE. Induction of fibrinolysis by venous obstruction. *Angiology* 1960; 11:367.
- Cole ER & Bachmann FW. Purification and properties of a plasminogen activator from pig heart. *J Biol Chem* 1977; 252:3729.
- Collen E. On the regulation and control of fibrinolysis. Edward Kowalski Memorial Lecture. *Thrombos Haemostas* 1980; 43:77.
- Collen D, Schetz J, De Cock F et al. Metabolism of antithrombin III (heparin cofactor) in man: effects of venous thrombosis and of heparin administration. *Eur J Clin Invest* 1977; 7:27.
- Colucci M, Paramo JA & Collen D. Generation in plasma of a fast-acting inhibitor of plasminogen activator in response to endotoxin stimulation. *J Clin Invest* 1985; 75:818.
- Colucci M, Stassen JM & Collen D. No influence of activated protein C (PCa) on the release of plasminogen activator (PA) in squirrel monkeys. *Haemostasis* 1984; 14:92 (abstract).
- Comp PC & Esmon CT. Generation of fibrinolytic activity by infusion of activated protein C into dogs. *J Clin Invest* 1981; 68:1221.
- Comp PC & Esmon CT. Recurrent venous thromboembolism in patients with a partial deficiency of protein S. *N Engl J Med* 1984; 311:1525.
- Comp PC, Nixon RR & Esmon CT. Determination of functional levels of protein C, an antithrombotic protein, using thrombin-thrombomodulin complex. *Blood* 1984; 63:15.
- Conard J, Brosstad F, Lie Larsen M et al. Molar antithrombin concentration in normal human plasma. *Haemostasis* 1983; 13:363.
- Conard J, Cazenave B, Samama M et al. AT III content and antithrombin activity in oestrogen-progestogen and progestogen-only treated women. *Thromb Res* 1980; 18:675.
- Coughlin SR, Moskowitz MA, Zetter BR et al. Platelet-dependent stimulation of prostacyclin synthesis by platelet-derived growth factor. *Nature* 1980; 288:600.
- Crowle AJ. Immunodiffusion. Academic Press, New York, San Francisco, London 1973; 2nd ed. p. 361-364 and 393-399.
- Dahlbäck B & Stenflo J. The activation of prothrombin by platelet-bound factor Xa. *Eur J Biochem* 1980; 104:549.
- Dahlbäck B & Stenflo J. High molecular weight complex in human plasma between vitamin K-dependent protein S and complement component C4b-binding protein. *Proc Natl Acad Sci* 1981; 78:2512.
- Danielsson Å & Björk I. Properties of antithrombin-thrombin complex formed in the presence and in the absence of heparin. *Biochem J* 1983; 213:345.
- Davie EW, Fujikawa K, Kurachi K & Kisiel W. The role of serine proteases in the blood coagulation cascade. *Adv Enzymol* 1979; 48:277.

- De Swiet M, Dorrington Ward P, Fidler J et al. Prolonged heparin therapy in pregnancy causes bone demineralization. *Br J Obstet Gynaecol* 1983; 90:1129.
- DiScipio RG and Davie EW. Characterization of protein S, a γ -carboxyglutamic acid containing protein from bovine and human plasma. *Biochemistry* 1979; 18:899.
- Edlund T, Ny T, Rånby M et al. Isolation of cDNA sequences coding for a part of human tissue plasminogen activator. *Proc Natl Acad Sci* 1983; 80:349.
- Egeberg O. Inherited antithrombin deficiency causing thrombophilia. *Thromb Diathes Haemorrh* 1965; 13:516.
- Emeis JJ, van Hinsbergh VWM, Verheijen JH & Wijngaards G. Inhibition of tissue-type plasminogen activator by conditioned medium from cultured human and porcine vascular endothelial cells. *Biochem Biophys Acta* 1983; 110:392.
- Erickson LA, Ginsberg MH & Loskutoff DJ. An inhibitor of plasminogen activator in human platelets. *Haemostasis* 1984; 14:39 (abstract).
- Esmon CT, Esmon NL & Harris KW. Complex formation between thrombin and thrombomodulin inhibits both thrombin-catalyzed fibrin formation and factor V activation. *J Biol Chem* 1982; 257:7944.
- Fagerhol MK & Abildgaard U. Immunological studies on human antithrombin III. Influence of age, sex and use of oral contraceptives on serum concentration. *Scand J Haematol* 1970; 7:10.
- Feinstein DI & Rapaport SI. Acquired inhibitors of blood coagulation. In: *Progress in Hemostasis and Thrombosis* (ed. TH Spaet). Grune and Stratton, New York 1972, p. 75.
- Ferguson WS & Finlay TH. Localization of the disulfide bond in human antithrombin III required for heparin-accelerated thrombin inactivation. *Arch Biochem Biophys* 1983; 221:304.
- Filip DJ, Eckstein JD & Veltkamp JJ. Hereditary antithrombin III deficiency and thromboembolic disease. *Am J Hematol* 1976; 2:343.
- Francis Jr RB & Patch MJ. A functional assay for protein C in human plasma. *Thromb Res* 1983; 32:605.
- Fredin H, Nilsson B, Rosberg B & Tengborn L. Pre- and postoperative levels of antithrombin III with special reference to thromboembolism after total hip replacement. *Thromb Haemostas* 1983; 49:158.
- Friberger P, Knös M, Gustavsson S et al. Methods for determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. *Haemostasis* 1978; 7:138.
- Gader AMA, da Costa J & Cash JD. A new vasopressin analogue and fibrinolysis. *Lancet* 1973; II:1417.
- Gaston LW. Studies on a family with an elevated level of factor V (pro-accelerin) and a tendency to thrombosis. *J Pediat* 1966; 68:367.
- Girolami A, Pengo V, Cappellato G et al. Antithrombin III Padua: A "new" congenital antithrombin III abnormality with normal or near normal activity, normal antigen, abnormal migration and no thrombotic disease. *Folia Haematol* 1983; 110:111.
- Glover JS, Salter DN & Shepherd BP. A study of some factors that influence the iodination of ox insulin. *Biochem J* 1967; 103:120.

- Gomperts ED, Feeseey M & van der Walt JD. Two dimensional immunoelectrophoretic studies in anti-thrombin III deficiency. *Thromb Res* 1976; 8:713.
- Granelli-Piperno A & Reich E. A study of proteases and protease-inhibitor complexes in biological fluids. *J Exp Med* 1978; 148:223.
- Griffin JH, Evatt B, Zimmerman T et al. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 1981; 68:1370.
- Griffith GC, Nichols G, Asher JD & Hanagan B. Heparin osteoporosis. *J Am Med Ass* 1965; 193:91.
- Gurewich V, Pannell R, Louise S et al. Effective and fibrin-specific clot lysis by a zymogen precursor form of urokinase (pro-urokinase). A study by in vitro and in two animal species. *J Clin Invest* 1984; 73:1731.
- Gyzander E, Eriksson E & Teger-Nilsson A-C. Determination of tissue plasminogen activator in plasma after adsorption on lysine-Sepharose. In: *Progress in Fibrinolysis* (ed. JF Davidson, F Bachmann, CA Bouvier & EKO Kruithof). Churchill Livingstone, London 1983, vol. VI, p. 425.
- Haglund O, Wibell L & Saldeen T. Plasminogen activators and inhibitors of plasminogen activator after venous occlusion in patients with deep venous thrombosis (DVT). *Haemostasis* 1984; 14:102 (abstract).
- Hamberg M, Svensson J & Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci* 1975; 72:2994.
- Harvey S, Minowada J, Takita H et al. Urokinase-like plasminogen activators of unusually high molecular weight secreted by a cell line derived from a human lung cancer case. *J Biol Chem* 1982; 257:5645.
- Hauert J & Bachmann F. Single-chain form of urokinase in human plasma. *Haemostasis* 1984; 14:15 (abstract).
- Hedner U & Martinsson G. Inhibition of activated Hageman factor (factor XIIa) by an inhibitor of the plasminogen activation (PA inhibitor). *Thromb Res* 1978; 12:1015.
- Hedner U & Nilsson IM. Antithrombin III in a clinical material. *Thromb Res* 1973; 3:631.
- Hedner U & Nilsson IM. The role of fibrinolysis. *Clin Haematol* 1981; 10:327.
- Hedner U, Nilsson IM, Bergentz S-E & Cronberg L. Hyperactive connective tissue in seven patients with recurrent thrombotic occlusions. *Haemostasis* 1973; 1:148.
- Heimark RL, Kurachi K, Fujikawa K & Davie EW. Surface activation of blood coagulation, fibrinolysis and kinin formation. *Biochemistry* 1980; 286:456.
- Heimark RL & Schwartz SM. Binding of coagulation factors IX and X to the endothelial cell surface. *Biochem Biophys Res Comm* 1983; 111:723.
- Heldin CH, Westermarck B & Wasteson A. Platelet-derived growth factor. Isolation by a large-scale procedure and analysis of subunit composition. *Biochem J* 1981; 193:907.
- Hellem AJ. The adhesiveness of human blood platelets in vitro. *Scand J Clin Lab Invest* 1960; 12 (Suppl 51):117.
- Hellgren M & Nygård EB. Blood coagulation and fibrinolysis in fertile women with previous thromboembolic complications and effects of venous occlusion. *Thromb Res* 1981; 24:453.

- Hellgren M, Tengborn L & Abildgaard U. Pregnancy in women with congenital antithrombin III deficiency: Experience of treatment with heparin and antithrombin. *Gynecol Obstet Invest* 1982; 14:127.
- Henschen A & Lottspiech F. Structural characteristics and plasmin susceptibility of the different molecular weight forms of urokinase. In: *Progress in Fibrinolysis* (ed. JF Davidson, F Bachmann, CA Bouvier & EKO Kruit-hof). Churchill Livingstone, London 1983, vol VI, p. 344.
- Holmberg L, Bladh B & Åstedt B. Purification of urokinase by affinity chromatography. *Biochim Biophys Acta* 1976; 445:215.
- Holmberg L, Kristoffersson A-C, Lecander I et al. Immunoradiometric quantification of tissue plasminogen activator secreted by fetal organs. Comparison with urokinase. *Scand J Clin Lab Invest* 1982a; 42:347.
- Holmberg L & Nilsson IM. AHF related protein in clinical praxis. *Scand J Haematol* 1973; 12:221.
- Holmberg L, Nilsson IM, Wallén P & Åstedt B. Studies of the blood plasminogen activator induced by 1-desamino-8-D-arginine vasopressin with observations in von Willebrand's disease. *Proc Soc Exp Biol Med* 1982b; 170:126.
- Holmer E. Mekanismer för heparinets effekt på inhibitionen av olika koagulationsfaktorer. In: *Heparin, struktur, biologisk funktion och klinisk användning*. Erik Jorpes Symposium 1981 (red. M. Blombäck). Svenska Läkaresällskapets Handlingar Hygiea 1982, 91:25.
- Holmer E, Kurachi K & Söderström G. The molecular-weight dependence of the rate-enhancing effect of heparin on the inhibition of thrombin, factor Xa, factor IXa, factor XIa, factor XIIa and kallikrein by antithrombin. *Biochem J* 1981; 193:395.
- Holmer E, Mattsson C & Nilsson S. Anticoagulant and antithrombotic effects of heparin and low molecular weight heparin fragments in rabbits. *Thromb Res* 1982; 25:475.
- Holmsen H. Biochemistry of the platelet release reaction. In: *Biochemistry and Pharmacology of Platelets*. Ciba Foundation Symposium. 35, Elsevier, North Holland Biomedical Press, Amsterdam 1975, p. 175.
- Holmsen H & Weiss HJ. Secretable storage pools in platelets. *Ann Rev Med* 1979; 30:119.
- Höök M, Björk I, Hopwood J & Lindahl U. Anticoagulant activity of heparin: separation of high-activity and low-activity heparin species by affinity chromatography on immobilized antithrombin. *FEBS Letters* 1976; 66:90.
- Hoylaerts M, Owen WG & Collen D. Involvement of heparin chain length in the heparin-catalyzed inhibition of thrombin by antithrombin III. *J Biol Chem* 1984; 259:5670.
- Hume M, Sevitt S & Thomas LP. Venous thrombosis and pulmonary embolism. Harvard Univ Press, Cambridge 1970.
- Isacson S & Nilsson IM. Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. *Acta Chir Scand* 1972; 138:313.
- Jespersen J, Rasmussen NR & Toftgaard C. Observations during the treatment with antithrombin-III concentrate of a case of tampon-related toxic shock syndrome and disseminated intravascular coagulation. Discrepancies between functional and immunologic determinations of antithrombin. *Thromb Res* 1982; 26:457.

- Johansson L, Nylander G, Hedner U & Nilsson IM. Comparison of streptokinase with heparin: Late results in the treatment of deep venous thrombosis. *Acta Med Scand* 1979; 206:93.
- Jordan R, Beeler D & Rosenberg R. Fractionation of low molecular weight heparin species and their interaction with antithrombin. *J Biol Chem* 1979; 254:2902.
- Jørgensen M, Mortensen JZ, Madsen AG et al. A family with reduced plasminogen activator activity in blood associated with recurrent venous thrombosis. *Scand J Haematol* 1982; 29:217.
- Juhan-Vague I, Moerman B, De Cock F et al. Plasma levels of a specific inhibitor of tissue-type plasminogen activator (and urokinase) in normal and pathological conditions. *Thromb Res* 1984; 33:523.
- Kaplan AP. Initiation of the intrinsic coagulation and fibrinolytic pathways in man. The role of surfaces, Hageman factor, prekallikrein, high molecular weight kininogen and factor XI. In: *Progress in Haemostasis and Thrombosis* (ed. TH Spaet). Grune & Stratton Inc, New York 1978, vol. 4, p. 127.
- Kaplan KL, Broekman MJ, Chernoff A et al. Platelet α -granule proteins: Studies on release and subcellular localization. *Blood* 1979; 53:604.
- Kauffman RH, Graeff JD, de la Rivière GB & van Es LA. Unilateral renal vein thrombosis and nephrotic syndrome. *Am J Med* 1976; 60:1048.
- Kauffman RH, Velthkamp JJ, van Tilburg NH & van Es LA. Acquired antithrombin III deficiency and thrombosis in the nephrotic syndrome. *Am J Med* 1978; 65:607.
- von Kaulla E & von Kaulla KN. Deficiency of antithrombin III activity associated with hereditary thrombosis tendency. *J Med* 1972; 3:349.
- Kisiel W. Human plasma protein C. Isolation, characterization, and mechanism of activation by α -thrombin. *J Clin Invest* 1979; 64:761.
- Kisiel W, Fujikawa K & Davie EW. Activation of bovine factor VII (proconvertin) by factor XII_a (activated Hageman factor). *Biochemistry* 1977; 16:4189.
- Kjeldgaard A & Larsson B. Fibrinolytic activity in the walls of foot veins in women using combined contraceptive pills. *Gynecol Obstet Invest* 1983; 15:223.
- Kluft C. Quantitation and behaviour of extrinsic or vascular plasminogen activator in blood. In: *Progress in Fibrinolysis* (ed. JF Davidson, IM Nilsson & B Åstedt). Churchill Livingstone, London 1981, vol. V, p. 24.
- Kluft C, Trumpi-Kalshoven MM, Jie AFH & Veldhuyzen-Stolk EC. Factor XII-dependent fibrinolysis: A double function of plasma kallikrein and the occurrence of a previously undescribed factor XII- and kallikrein-dependent plasminogen proactivator. *Thrombos Haemostas* 1979; 41:756.
- Kluft C, Verheijen JH, Cooper P et al. Post-operative changes in the activity in blood of extrinsic (tissue-type) plasminogen activator and its fast-acting inhibitor. *Haemostasis* 1984; 14:26 (abstract).
- Koide T, Odani S, Takahashi K et al. Antithrombin III Toyama: Replacement of arginine-47 by cysteine in hereditary abnormal antithrombin III that lacks heparin-binding ability. *Proc Natl Acad Sci* 1984; 81:289.
- Kok P & Astrup T. Isolation and purification of a tissue plasminogen activator and its comparison with urokinase. *Biochemistry* 1969; 8:79.
- Korninger C & Collen D. Studies on the specific fibrinolytic effect of human extrinsic (tissue-type) plasminogen activator in human blood and in various animal species in vitro. *Thrombos Haemostas* 1981; 46:561.

- Kruithof EKO, Tran-Thang C, Ransijn A & Bachmann F. Demonstration of a fast-acting inhibitor of plasminogen activators in human plasma. *Blood* 1984; 64:907.
- Kruse-Blinkenberg HO, Gormsen J, Jensen J et al. Low dose heparin in elective abdominal surgery. *Acta Chir Scand* 1980; 146:383.
- Kucinski CS, Fletcher AP & Sherry S. Effect of urokinase antiserum on plasminogen activators: demonstration of immunologic dissimilarity between plasma plasminogen activator and urokinase. *J Clin Invest* 1968; 47:1238.
- Lam LH, Silbert JE & Rosenberg RD. The separation of active and inactive forms of heparin. *Biochem Biophys Res Comm* 1976; 69:570.
- Lane JL, Bird P & Rizza CR. A new assay for the measurement of total progressive antithrombin. *Br J Haematol* 1975; 30:103.
- Laurell C-B. Electro-immunoassay. *Scand J Clin Lab Invest* 1972; 29 (Suppl 124):21.
- Laurent TC, Tengblad A, Thunberg L et al. The molecular-weight-dependence of the anti-coagulant activity of heparin. *Biochem J* 1978; 175:691.
- Laursen B, Mortensen JZ, Frost L & Hansen KB. Disseminated intravascular coagulation in hepatic failure treated with antithrombin III. *Thromb Res* 1981; 22:701.
- Lechner K, Tahler E, Niessner H et al. Antithrombin-III-Mangel und Thrombose-
heigung. *Wien Klin Wschr* 1977; 89:215.
- Lee AKY, Chan V & Chan TK. The identification and localization of antithrombin III in human tissues. *Thromb Res* 1979; 14:209.
- Léon M, Aiach M, Guennec J-Y et al. Antithrombin III in rat hepatocytes. *Thromb Res* 1982; 28:115.
- Leone G, Cotumaccio R, de Stefano V & Zanetti L. Antithrombin III Roma: A familial quantitative-qualitative AT-III deficiency identifiable by crossed immunoelectrophoresis and by crossed immunoelectrophoresis. *Haematologica* 1983; 68:765.
- Letsky EA & De Swiet M. Thromboembolism in pregnancy and its management. *Br J Haematol* 1984; 57:543.
- Levin EG. Latent tissue plasminogen activator produced by human endothelial cells in culture: Evidence for an enzyme-inhibitor complex. *Proc Natl Acad Sci* 1983; 80:6804.
- Levin EG & Loskutoff DJ. Comparative studies of the fibrinolytic activity of cultured vascular cells. *Thromb Res* 1979; 15:869.
- Lindahl U & Höök M. Glycosaminoglycans and their binding to biological macromolecules. *Ann Rev Biochem* 1978; 47:385.
- Ljungrén H, Bergqvist D, Isacson S & Nilsson IM. Comparison between the plasminogen activator activity in walls of superficial, muscle and deep veins. *Thromb Res* 1981; 22:295.
- Ljungrén H, Holmberg L, Kjeldgaard A et al. Immunological characterisation of plasminogen activators in the human vessel wall. *J Clin Pathol* 1983; 36:1046.
- Loskutoff DJ & Edgington TS. Synthesis of a fibrinolytic activator and inhibitor by endothelial cells. *Proc Natl Acad Sci* 1977; 74:3903.
- Loskutoff DJ, van Mourik JA, Erickson LA & Lawrence D. Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. *Proc Natl Acad Sci* 1983; 80:2956.
- McEntee MP, Jesty J & Hultin MB. Effect of oral contraceptives on antithrombin III. *Thromb Res* 1981; 24:13.

- McFarlane AS. Metabolism of plasma proteins. In: Mammalian Protein Metabolism I (ed. HN Munro & JB Allison). Academic Press, New York, London 1964, p. 297.
- Macfarlane RG & Pilling J. Fibrinolytic activity of normal urine. *Nature* 1947; 159:779.
- McKay EJ. A simple two-step procedure for the isolation of antithrombin III from biological fluids. *Thromb Res* 1981; 21:375.
- McLean J. The thromboplastic action of cephalin. *Am J Physiol* 1916; 41:250.
- Mammen EF. Fibrinogen abnormalities. *Semin Thromb Hemostas* 1983; 9:1.
- Mancini G, Carbonara AO & Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J Immunochem* 1965; 2:235.
- Mannucci PM, Boyer C, Wolf CBM et al. Treatment of congenital antithrombin III deficiency with concentrates. *Br J Haematol* 1982; 50:531.
- Manotti C, Quintavalla R, Megha A et al. Inherited deficiency of antithrombin III in two Italian families. *Haemostasis* 1982; 12:300.
- Marciniak E, Farley CH & DeSimone PA. Familial thrombosis due to antithrombin III deficiency. *Blood* 1974; 43:219.
- Marciniak E & Gockerman JP. Heparin-induced decrease in circulating antithrombin-III. *Lancet* 1977; 2:581.
- Marcum JA & Rosenberg D. Heparinlike molecules with anticoagulant activity are synthesized by cultured endothelial cells. *Biochem Biophys Res Comm* 1985; 126:365.
- Marcus AJ. Platelet aggregation. In: Hemostasis and Thrombosis (ed. RW Colman, J Hirsh, VJ Marder & EW Salzman). J.B. Lippincott Company, Philadelphia, Toronto 1982, p. 380.
- Marlar RA & Griffin JH. Deficiency of protein C inhibitor in combined factor V/VIII deficiency disease. *J Clin Invest* 1980; 66:1186.
- Marlar RA, Kleiss AJ & Griffin JH. Modes of anticoagulant action of human protein C, a vitamin K dependent serine protease. *Fed Proc* 1980; 39:544.
- van der Meer J, Stoepman-van Dalen EA & Jansen JMS. Antithrombin-III deficiency in a Dutch family. *J Clin Pathol* 1973; 26:532.
- Mettinger KL. A study of hemostasis in ischemic cerebrovascular disease. I. Abnormalities in factor VIII and antithrombin. *Thromb Res* 1982; 26:183.
- Mettinger KL & Egberg N. A study of hemostasis in ischemic cerebrovascular disease. III. Abnormalities in vascular plasminogen activators, anti-activators and α_2 -antiplasmin. *Thromb Res* 1982; 26:203.
- Miletich JP, Jackson CM & Majerus PW. Interaction of coagulation Xa with human platelets. *Proc Natl Acad Sci* 1977; 74:4033.
- Miller-Andersson M, Borg H & Andersson L-O. Purification of antithrombin III by affinity chromatography. *Thromb Res* 1974; 5:439.
- Moncada S & Vane JR. Unstable metabolites of arachidonic acid and their role in hemostasis and thrombosis. *Br Med Bull* 1978; 34:129.
- Moore S, Pepper DS & Cash JD. The isolation and characterization of platelet specific β -globulin (β -thromboglobulin) and the detection of anti-urokinase and anti-plasmin released from thrombin-aggregated washed human platelets. *Biochim Biophys Acta* 1975a; 379:360.
- Moore S, Pepper DS & Cash JD. Platelet antiheparin activity. The isolation and characterisation of platelet factor 4 released from thrombin-aggregated washed human platelets and its dissociation into subunits and the isolation of membrane-bound antiheparin activity. *Biochim Biophys Acta*

1975b; 379:370.

- Morawitz P. Die Chemie der Blutgerinnung. *Ergebn Physiol* 1905; 4:308.
- Moritz MW, Sutura SP & Joist JH. Factors influencing shear-induced platelet alterations: Platelet lysis is independent of platelet aggregation and release. *Thromb Res* 1981; 22:445.
- Mosvold J, Abildgaard U, Jenssen H & Andersson R. Low antithrombin III in acute hepatic failure at term. *Scand J Haematol* 1982; 29:48.
- Müllertz S & Clemmensen I. The primary inhibitor of plasmin in human plasma. *Biochem J* 1976; 159:545.
- Müllertz S & Lassen M. An activator system in blood indispensable for the formation of plasma by streptokinase. *Proc Soc Exp Biol Med* 1953; 82:264.
- Murano G. The "Hageman" connection: Interrelationships of blood coagulation, fibrino(geno)lysis, kinin generation, and complement activation. *Am J Hematol* 1978; 4:409.
- Mustard JF, Kinlough-Rathbone RL & Packham MA. The vessel wall in thrombosis. In: *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*. JB Lippincott Company, Philadelphia, Toronto 1982, p. 703.
- Nagy I & Losonczy H. Three types of hereditary antithrombin III deficiency. *Thrombos Haemostas* 1979; 42:187 (abstract).
- Nicolaidis AN, Clark CT, Thomas RD & Lewis JD. Soleal veins and local fibrinolytic activity. *Br J Surg* 1972; 59:914.
- Niewiarowski S. Proteins secreted by the platelet. *Thrombos Haemostas* 1977; 38:924.
- Niewiarowski S & Prou-Wartelle O. Role du facteur contact (facteur Hageman) dans la fibrinolyse. *Thromb Diathes Haemorrh* 1959; 3:593.
- Nilsson IM. *Haemorrhagic and Thrombotic Diseases*. John Wiley & Sons, London 1974.
- Nilsson IM. Coagulation, fibrinolysis and venous thrombosis. *Triangle* 1977;16:19.
- Nilsson IM, Felding P, Lecander I et al. Different types of plasminogen activator inhibitors in plasma and platelets in pregnant women. *Br J Haematol* 1985a; 61 (in press).
- Nilsson IM, Krook H, Sternby N-H et al. Severe thrombotic disease in a young man with bone marrow and skeletal changes and with a high content of an inhibitor in the fibrinolytic system. *Acta Med Scand* 1961; 169:323.
- Nilsson IM, Ljungnér H & Tengborn L. Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. *Br Med J* 1985b; 290:1453.
- Nilsson IM & Olow B. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 1962; 123:247.
- Nilsson T, Bergsdorf N & Wallén P. Tissue-type plasminogen activator in patients with idiopathic thromboembolic disease as measured by a new ELISA. In: *Clinical Aspects of Fibrinolysis and Thrombolysis* (ed. J Jespersen, C Kluft & O Korsgaard). South Jutland University Press, Esbjerg, Danmark 1983; p. 95.
- Noordhoek-Hegt V & Brakman P. Histochemical study of an inhibitor of fibrinolysis in the human arterial wall. *Nature* 1974; 248:75.
- Nordenman B, Nyström C & Björk I. The size and shape of human and bovine antithrombin III. *Eur J Biochem* 1977; 78:195.
- Nosslin B. Mathematical model of plasma protein turnover determined with ¹³¹I-labelled protein. In: *Metabolism of Human Gamma Globulin (γ_{ss} globulin)*

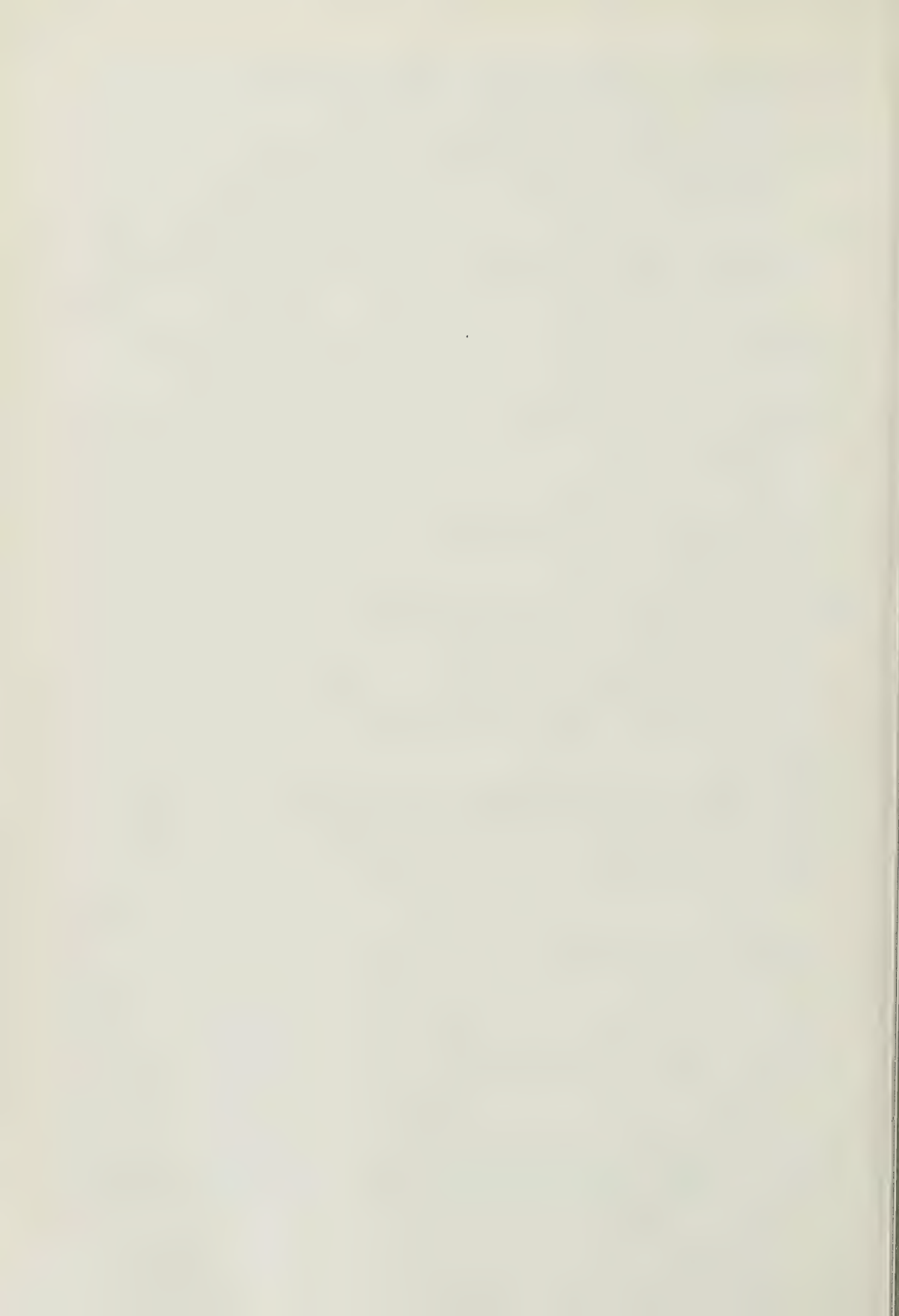
- (ed. SB Andersen). Blackwell Scientific Publications, Oxford 1964, p.115.
- Ødegård OR, Lie M & Abildgaard U. Heparin cofactor activity measured with an amidolytic method. *Thromb Res* 1975; 6:287.
- Ødegård OR, Lie M & Abildgaard U. Antifactor Xa activity measured with amidolytic methods. *Haemostasis* 1976; 5:265.
- Østerud B & Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: Additional pathway for initiating blood coagulation. *Proc Natl Acad Sci* 1977; 74:5260.
- Owen WG. The control of hemostasis. Role of endothelium in the regulation of inhibitory and catabolic pathways. *Arch Pathol Lab Med* 1982; 106:209.
- Pandolfi M, Bjørnstad A & Nilsson IM. Technical remarks on the microscopical demonstration of tissue plasminogen activator. *Thromb Diathes Haemorrh* 1972; 27:88.
- Pandolfi M, Hedner U & Nilsson IM. Bilateral occlusion of the retinal veins in a patient with inhibition of fibrinolysis. *Ann Ophthal* 1970; 2:481.
- Pandolfi M, Nilsson IM, Robertson B & Isacson S. Fibrinolytic activity of human veins. *Lancet* 1967; 2:127.
- Paraskevas N, Nilsson IM & Martinsson G. A method for determining serum inhibitors of plasminogen activation. *Scand J Clin Lab Invest* 1962; 14:138.
- Penick GD, Dejanov JJ, Reddick RL & Roberts HR. Predisposition to intravascular coagulation. *Thromb Diathes Haemorrh* 1966; 21:543.
- Pennica D, Holmes WE, Kohr WJ et al. Cloning and expression of human tissue-type plasminogen activator cDNA in E.coli. *Nature* 1983; 301:214.
- Petersen TE, Dudek-Wojciechowska G, Sottrup-Jensen L & Magnusson S. Primary structure of antithrombin-III (heparin cofactor). Partial homology between α_1 -antitrypsin and antithrombin-III. In: *Physiological Inhibitors of Coagulation and Fibrinolysis* (ed. D Collen, B Wiman & M Verstraete). Elsevier/North Holland Biomedical Press 1979; p. 43.
- Philips M, Juul A-G & Thorsen S. Human endothelial cells produce a plasminogen activator inhibitor and a tissue-type plasminogen activator-inhibitor complex. *Biochem Biophys Acta* 1984; 802:99.
- Prydz H. Vitamin K-dependent clotting factors. *Sem Thromb Hemostas* 1977; 4:1.
- Prydz H. The role of factor VII in the intrinsic pathway: A brief review. *Haemostasis* 1983; 13:156.
- Rákóczi I, Wiman B & Collen D. On the biological significance of the specific interaction between fibrin, plasminogen and antiplasmin. *Biochim Biophys Acta* 1978; 540:295.
- Rånby M. Studies on the kinetics of plasminogen activation by tissue plasminogen activator. *Biochim Biophys Acta* 1982; 704:461.
- Rånby M & Wallén P. A sensitive parabolic rate assay for the tissue plasminogen activator. In: *Progress in Fibrinolysis* (ed. JF Davidson, IM Nilsson & B Åstedt). Churchill Livingstone, London 1981; vol. V, p. 233.
- Ratnoff OD. A quarter century with Mr. Hageman. *Thrombos Haemostas* 1980; 93:95.
- Reich E. Plasminogen activator: secretion by neoplastic cells and macrophages. In: *Proteases and Biological Control* (ed. E Reich, DB Rifkin & E Shaw). Cold Spring Harbor Laboratory, New York 1975, p. 333.
- Rickli EE & Otavsky WI. A new method of isolation and some properties of the heavy chain of human plasmin. *Eur J Biochem* 1975; 59:441.
- Rifkin DB, Loeb JN, Moore G & Reich E. Properties of plasminogen activators formed by neoplastic human cell cultures. *J Exp Med* 1974; 139:1317.

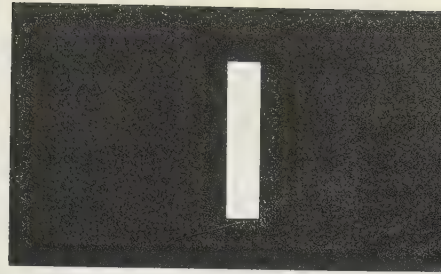
- Rijken DC & Collen D. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. *J Biol Chem* 1981; 256:7035.
- Rijken DC, Juhan-Vague I, De Cock F & Collen D. Measurement of human tissue-type plasminogen activator by a two-site immunoradiometric assay. *J Lab Clin Med* 1983; 101:274.
- Rijken DC, Wijngaards G & Welbergen J. Biochemical and immunological characterization of plasminogen activator from human tissue. In: *Progress in Chemical Fibrinolysis and Thrombolysis* (ed. JF Davidson, V Cepelak, M Samama & P Desnoyers). Churchill Livingstone, London 1979, vol. IV, p. 349.
- Rijken DC, Wijngaards G & Welbergen J. Relationship between tissue plasminogen activator and the activators in blood and vascular wall. *Thromb Res* 1980; 18:815.
- Robbins KC. The regulation and control of the blood fibrinolytic system. In: *Progress in Fibrinolysis* (ed. JF Davidson, IM Nilsson & B Åstedt). Churchill Livingstone, London 1981, vol V, p. 3.
- Robertson BR, Pandolfi M & Nilsson IM. "Fibrinolytic capacity" in healthy volunteers as estimated from effect of venous occlusion of arms. *Acta Chir Scand* 1972; 138:429.
- Rosenberg RD. Actions and interactions of antithrombin and heparin. *N Engl J Med* 1975; 292:146.
- Rosenberg RD & Damus PS. The purification and mechanism of action of human antithrombin-heparin cofactor. *J Biol Chem* 1973; 248:6490.
- Ross R & Vogel A. The platelet-derived growth factor. *Cell* 1978; 14:203.
- Rucinski B, Niewiarowski S, James P et al. Antiheparin proteins secreted by human platelets. Purification, characterization, and radioimmunoassay. *Blood* 1979; 53:47.
- Sagar S, Stamatakis JD, Thomas DP & Kakkar VV. Oral contraceptives, antithrombin-III activity, and postoperative deep-vein thrombosis. *Lancet* 1976; 1:509.
- Sakariassen KS, Bolhuis PA & Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-von Willebrand factor bound to the subendothelium. *Nature* 1979; 279:636.
- Sakuragawa N, Takahashi K, Kondo S & Koide T. Antithrombin III Toyama: A hereditary abnormal antithrombin III of a patient with recurrent thrombophlebitis. *Thromb Res* 1983; 31:305.
- Sala N, Owen WG & Collen D. A functional assay of protein C in human plasma. *Blood* 1984; 63:671.
- Samuelsson B, Goldyne M, Granström E et al. Prostaglandins and thromboxanes. *Ann Rev Biochem* 1978; 47:997.
- Sandberg H. Platelet factor 3. The link between platelets and plasma coagulation. Uppsala, Sweden 1979 (Thesis).
- Sandberg H & Andersson L-O. A highly sensitive assay of platelet factor 3 using a chromogenic substrate. *Thromb Res* 1979; 14:113.
- Sas G. Congenital and acquired defects of antithrombin III. *La Ricerca Clin Med* 1984; 14:491.
- Sas G, Blaskó G, Bánhegyi D et al. Abnormal antithrombin III (antithrombin III 'Budapest') as a cause of a familial thrombophilia. *Thromb Diathes Haemorrh* 1974; 32:105.

- Sas G, Pepper DS & Cash JD. Further investigations on antithrombin III in the plasmas of patients with the abnormality of 'antithrombin III Budapest'. *Thromb Diathes Haemorrh* 1975; 33:564.
- Sas G, Pető I, Bánhegyi D et al. Heterogeneity of the "classical" antithrombin III deficiency. *Thrombos Haemostas* 1980; 43:133.
- Sasahara AA, Bell WR, Simon TL et al. The phase II urokinase - streptokinase pulmonary embolism trial. *Thromb Diathes Haemorrh* 1975; 33:464.
- Schmidt A. *Zur Blutlehre*, Leipzig F.C.W. Vogel 1892.
- Schwarz HP, Fischer M, Hopmeier P et al. Plasma protein S deficiency in familial thrombotic disease. *Blood* 1984; 64:1297.
- Scully MF, De Haas H, Chan P & Kakkar VV. Hereditary antithrombin III deficiency in an English family. *Br J Haematol* 1981; 47:235.
- Seegers WH, Warner ED, Brinkhous KM & Smith HP. Heparin and the anti-thrombotic activity of plasma. *Science* 1942; 96:300.
- Seligsohn U, Berger A, Abend M et al. Homozygous protein C deficiency manifested by massive venous thrombosis in the newborn. *N Engl J Med* 1984; 310:559.
- Semeraro N, Colucci M, Telesforo P & Collen D. The inhibition of plasmin by antithrombin-heparin complex. *Br J Haematol* 1978; 39:91.
- Shapiro SS & Thiagarajan P. Lupus anticoagulants. In: *Progress in Hemostasis and Thrombosis* (ed. TH Spaet). Grune & Stratton, New York 1982, vol. 6, p. 263.
- Sharma GVRK, Burleson VA & Sasahara AA. Effect of thrombolytic therapy on pulmonary-capillary blood volume in patients with pulmonary embolism. *N Engl J Med* 1980; 303:842.
- Sié P & Boneu B. Antithrombin III (AT III) requirement for the anticoagulant effect of unfractionated heparin (UH) in vitro. *Thrombos Haemostas* 1983; 50:161 (abstract).
- Smith RL, Blick EF, Coalson J & Stein PD. Thrombus production by turbulence. *J Appl Physiol* 1972; 82:261.
- Sørensen PJ, Sas G, Pető I et al. Distinction of two pathologic antithrombin III molecules: Antithrombin III 'Aalborg' and antithrombin III 'Budapest'. *Thromb Res* 1982; 26:211.
- Sottrup-Jensen L, Claeys H, Zajdel M et al. The primary structure of human plasminogen: Isolation of two lysine-binding fragments and one "mini"-plasminogen (MW, 38,000) by elastase-catalyzed-specific limited proteolysis. In: *Progress in Chemical Fibrinolysis and Thrombolysis* (ed. JF Davidson, RM Rowan, MM Samama & PC Desnoyers). Raven Press, New York 1978; vol. 3, p. 191.
- Spaet TH & Tsao CH. Vascular endothelium and thrombogenesis. In: *Thrombosis* (ed. S Sherry, KM Brinkhous, E Genton & JM Stengle). National Academy of Sciences, Washington DC 1969, p. 416.
- Spire B, Dormont D, Barre-Sinoussi F et al. Inactivation of lymphadenopathy-associated virus by heat, gamma rays, and ultraviolet light. *Lancet* 1985; 1:188.
- Stead BW, Bauer KA, Kinney TR et al. Venous thrombosis in a family with defective release of vascular plasminogen activator and elevated plasma factor VIII/von Willebrand's factor. *Am J Med* 1983; 74:33.
- Stenflo J. A new vitamin-K dependent protein. Purification from bovine plasma and preliminary characterization. *J Biol Chem* 1976; 251:355.

- Stern DM, Drillings M, Nossel HL et al. Binding of factors IX and IX_a to cultured vascular endothelial cells. *Proc Natl Acad Sci* 1983; 80:4119.
- Stewart GJ, Stern HR & Schaub RG. Endothelial alterations, deposition of blood elements and increased accumulation of ¹³¹I-albumin in canine jugular veins following abdominal surgery. *Thromb Res* 1978; 12:555.
- Sundqvist S-B, Hedner U, Kullenberg HKE & Bergentz S-E. Deep venous thrombosis of the arm; a study of coagulation and fibrinolysis. *Br Med J* 1981; 283:265.
- Suzuki K, Nishioka J & Hashimoto S. Protein C inhibitor. Purification from human plasma and characterization. *J Biol Chem* 1983; 258:163.
- Sveger T. Antithrombin III in adolescents. *Thromb Res* 1979; 15:885.
- Teger-Nilsson A-C, Friberger P & Gyzander E. Determination of a new rapid plasmin inhibitor in human blood by means of a plasmin specific tripeptide substrate. *Scand J Clin Lab Invest* 1977; 37:403.
- Tengborn L & Bengtsson T. Antithrombin III concentrate. *Acta Obstet Gynecol Scand* 1985; 64 (in press).
- Tengborn L, Larsson SA, Hedner U & Nilsson IM. Coagulation studies in children and young adults with cerebral ischemic episodes. *Acta Neurol Scand* 1981; 63:351.
- Thaler E, Balzar E, Kopsa H & Pinggera WF. Acquired antithrombin III deficiency in patients with glomerular proteinuria. *Haemostasis* 1978; 7:257.
- Thiagarajan P, Shapiro SS & De Marco L. Monoclonal immunoglobulin M λ coagulation inhibitor with phospholipid specificity. *J Clin Invest* 1980; 66:397.
- Thorell JI & Johansson BG. Enzymatic iodination of polypeptides with ¹²⁵I to high specific activity. *Biochim Biophys Acta* 1971; 215:363.
- Thorsen S. Human urokinase and porcine tissue plasminogen activator. *Danish Med Bull* 1977; 25:189.
- Thorsen S, Glas-Greenwalt P & Astrup T. Differences in the binding to fibrin of urokinase and tissue plasminogen activator. *Thromb Diathes Haemorrh* 1972; 28:65.
- Thorsen S & Philips M. Isolation of tissue-type plasminogen activator-inhibitor complexes from human plasma. Evidence for a rapid plasminogen activator inhibitor. *Biochim Biophys Acta* 1984; 802:111.
- Thunberg L, Bäckström C & Lindahl U. Further characterization of the anti-thrombin-binding sequence in heparin. *Carbohydrate Res* 1982; 100:393.
- Todd AS. Fibrinolysis autographs. *Nature* 1958; 181:495.
- Tran TH, Bondeli C, Marbert GA & Duckert F. Reactivity of a hereditary abnormal antithrombin III fraction in the inhibition of thrombin and factor Xa. *Thrombos Haemostas* 1980; 44:92.
- Tranzer JP & Baumgartner HR. Filling gaps in the vascular endothelium with blood platelets. *Nature* 1967; 216:1126.
- Verheijen JH, Chang GTG & Kluft C. Evidence for the occurrence of a fast-acting inhibitor for tissue-type plasminogen activator in human plasma. *Thrombos Haemostas* 1984; 51:392.
- Virchow R. *Gesammelte Abhandlungen zur Wissenschaftlichen Medizin*. Frankfurt a. Main 1856.
- Wahlberg T, Blombäck M & Övermark I. Blood coagulation studies in 45 patients with ischemic cerebrovascular disease and 44 patients with venous thromboembolic disease. *Acta Med Scand* 1980; 207:385.

- Wallén P. Activation of plasminogen with urokinase and tissue activator. In: Thrombosis and Urokinase (ed. R Paoletti & S Sherry). Academic Press, London, New York, San Francisco 1977, p. 91.
- Wallén P, Kok P & Rånby M. The tissue activator of plasminogen. In: Proceedings of the 11th FEBS Meeting, Copenhagen (ed. S Magnusson, M Ottesen, B Foltmann et al). Pergamon Press, Oxford 1977, p 127.
- Wallén P, Rånby M, Bergsdorf N & Kok P. Purification and characterization of tissue plasminogen activator: on the occurrence of two different forms and their enzymatic properties. In: Progress in Fibrinolysis (ed. JF Davidson, IM Nilsson & B Åstedt). Churchill Livingstone, London 1981, vol. V, p. 16.
- Walsh PN. Platelet coagulant activities and hemostasis. A hypothesis. Blood 1974; 43:597.
- Walsh PN. Platelets and coagulation proteins. Fed Proc 1981; 40:2086.
- Weber R & Osborn M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem 1969; 244:4406.
- Wessler S, Gitel SN, Wan LS & Pasternack BS. Estrogen-containing oral contraceptive agents. A basis for their thrombogenicity. JAMA 1976; 236:2179.
- Wiman B, Csemiczky G, Marsk L & Robbe H. The fast inhibitor of tissue plasminogen activator in plasma during pregnancy. Thrombos Haemostas 1984; 52:124.
- Wiman B & Wallén P. The specific interaction between plasminogen and fibrin. A physiological role of the lysine binding site in plasminogen. Thromb Res 1977; 1:213.
- Winter JH, Bennett B, Watt JL et al. Confirmation of linkage between anti-thrombin III and Duffy blood group and assignment of AT3 to 1q22 → q25. Ann Hum Genet 1982; 46:35.
- Winter JH & Douglas AS. Familial venous thrombosis. Postgrad Med J 1983; 59:677.
- Wolf M, Boyer C, Lavergne JM & Larrieu MJ. A new familial variant of anti-thrombin III: 'Antithrombin III Paris'. Br J Haematol 1982; 51:285.
- Wolf M, Boyer C, Tripodi A et al. Antithrombin Milano. A new variant with monomeric and dimeric inactive antithrombin III. Blood 1985; 65:496.





Familial Antithrombin III Deficiency as Pathogenesis of Deep Venous Thrombosis

Lilian Johansson, Ulla Hedner and Inga Marie Nilsson

From the Coagulation Laboratory, Malmö General Hospital, Malmö, Sweden

ABSTRACT. A family including 18 members with decreased antithrombin III (AT III), measured with both a biological and an immunochemical method, is described. The pattern found on crossed immunoelectrophoresis, using heparin in the agarose in the first run, was normal, though the peaks were low. This suggests decreased synthesis of a normal protein in the affected members. AT III deficiency occurred in both the paternal and the maternal branch of the propositus. Six, all belonging to the maternal branch, of the above 18 persons had had at least one thromboembolic episode. Some of the episodes had been precipitated by the presence or occurrence of some predisposing event or circumstance. This suggests the possible occurrence of a gene making some of the maternal family members more susceptible to certain trigger factors, such as surgery, infection, pregnancy, and the puerperium. The mode of inheritance filled all the criteria for autosomal dominant transmission. Prophylactic treatment, preferably oral anticoagulants and/or dextran, is recommended for all persons with a low AT III concentration in any situation known to increase the predisposition to thrombosis. The effect of heparin in these patients is impaired since the heparin co-factor, which is identical with AT III, is lowered.

Key words: Venous thrombosis, antithrombin III, antithrombin III deficiency.

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Familial thromboembolism associated with decreased antithrombin III (AT III) activity was first described by Egeberg in 1965 (6). AT III deficiency has since been described as the cause of familial deep venous thrombosis (DVT) by several authors (4, 9, 13, 15, 16, 22). AT III has been determined with both a biological and an immunochemical method (1, 3, 7). The correlation between the results obtained by the two methods is, as a rule, high (2, 9, 16). However, the biological method cannot be replaced by the immunochemical. This was

clearly exemplified by Sas et al. (22) in a family in which the AT III was normal according to the immunochemical method, but low when measured with the biological method. Their group also showed that further information about the AT III function and protein can be obtained with the crossed immunoelectrophoresis technique.

Hereditary AT III deficiency as a cause of familial DVT seems to be rare. At our laboratory we have detected only 4 families with the disorder since the beginning of routine determination of AT III in the late sixties (roughly 175 primary examinations a year). This paper reports an investigation of 44 members of the largest of these families. None of the examinations were performed earlier than 4 weeks after the end of an episode of DVT.

METHODS

The following determinations were made: platelet count, platelet adhesiveness, factor VIII activity, factor VIII related antigen, P & P (prothrombin + proconvertin + factor X), factor V, fibrinogen, fibrin/fibrinogen degradation products, plasminogen, euglobulin clot lysis time, fibrinolytic activity of resuspended euglobulin precipitate of plasma on unheated fibrin plates, α_2 -macroglobulin, inhibitors of plasminogen activation and fibrinolytic activity in superficial vein walls. The procedures are described elsewhere (5, 11, 12, 17-20, 26). *Fibrinolytic response to venous occlusion* of the arms was performed according to Robertson et al. (21); 95% confidence interval 310-380 mm². AT III was determined (a) *biologically* according to Abildgaard et al. (3) using heat defibrinated plasma, and (b) *immunochemically* according to Hedner and Nilsson (10) using the rocket method of Laurell (14). Antiserum against AT III was obtained from Behringwerke, Marburg/Lahn, West Germany. Reference range for both methods 75-120%. (c) *Crossed immunoelectrophoresis* with heparin in the agarose was performed according to Sas et al. (24) but with the following modifications: 10 ml of 1% agarose solution, containing 16.6 U heparin/ml agarose, were poured onto a glass plate (110×110 mm).

Abbreviations: AT III=antithrombin III, DVT=deep venous thrombosis.

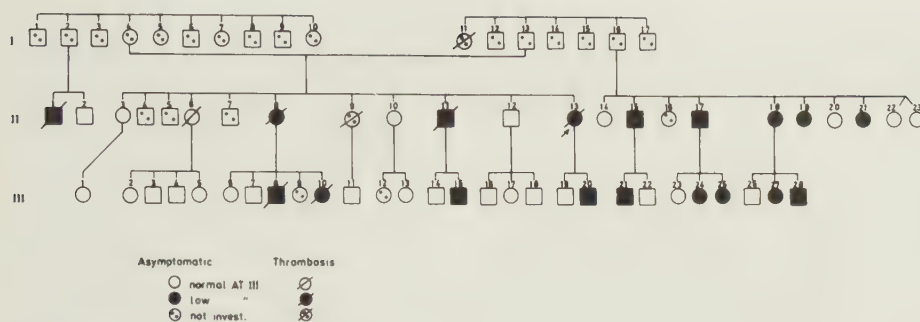


Fig. 1. Pedigree of the family.

After the agarose had set, a well 3 mm in diameter was cut out in one corner of the plate and 5 μ l of the sample was deposited into it. Electrophoresis was carried out at 20 mV/plate for 4 hours at room temperature and cooled with running tap water. After the first electrophoretic run the agarose gel was cut longitudinally into two parts. The cut was placed 25 mm from the edge of the plate. The smaller part of the agarose layer, which contained the separated proteins, was left untouched, while the large segment was

discarded and replaced by 7.5 ml of 1% agarose containing 1.5% anti-AT III immune serum. The plate was then turned 90° from the direction of the first run and electrophoresis was run with the plate in this direction at 10 mA overnight under the same conditions as the first run. The plate was then dried and stained with Coomassie brilliant blue. Normal, mixed plasma was always run simultaneously for comparison with the patient's plasma. When the AT III of the patient's plasma was low, the mixed plasma



Fig. 2. Crossed immunoelectrophoresis in agarose gel containing 16.6 U heparin/ml agarose. (a) Plasma from

II:1 (maternal relative), (b) from III:21 (paternal relative), (c and d) normal plasma.

Table I. Results of AT III determinations of the investigated family members according to biological (B) and immunochemical (I) methods (reference range 75–120 %)

Family member	Born in	DVT and/or pulmonary embolism	AT III (%)	
			B	I
Generation II				
1	1916	1	40	39
8	1919	>2	38	25
11	1929	1	41	34
13	1937	>2	45	38
15	1923	0	52	40
17	1927	0	49	55
18	1929	0	43	33
19	1930	0	43	46
21	1935	0	52	39
2	1920	0	129	108
3	1906	0	93	100
6	1913	1	116	86
10	1925	0	90	90
12	1932	0	124	104
14	1922	0	105	100
20	1933	0	86	120
22	1937	0	100	90
23	1937	0	92	72
Generation III				
8	1941	1	43	39
10	1955	1	50	43
15	1963	0	40	39
20	1964	0	53	35
21	1951	0	50	41
24	1965	0	43	37
25	1966	0	64	41
27	1956	0	58	50
28	1958	0	44	55
1	1932	0	82	62
2	1938	0	98	100
3	1945	0	127	124
4	1947	0	90	90
5	1950	0	150	124
6	1937	0	79	92
7	1939	0	90	100
11	1942	0	105	100
12	1947	0	90	68
14	1959	0	91	86
16	1960	0	98	100
17	1962	0	130	109
18	1963	0	143	100
19	1961	0	82	78
22	1953	0	82	100
23	1960	0	133	120
26	1951	0	100	100

was used both undiluted and diluted to the corresponding level of protein before the crossed immunoelectrophoresis was started.

Family study (Fig. 1 and Table I)

The propositus was a woman, born in 1937. At 13 years of age she had had pneumonia with clinical symptoms of

thrombosis of the left leg. Her first pregnancy in 1961 was complicated by thrombosis of the left leg and signs of recurrent pulmonary embolism. Her second child was born in 1964; pregnancy and parturition had been uncomplicated. In 1974 she had apparently had spontaneous, phlebographically verified thrombosis of the left leg on two occasions within about 6 weeks. She was treated with heparin and after her second spell in hospital she continued treatment with Waran® (warfarin sodium). Regular follow-up with Thrombotest® showed values within therapeutic ranges, but although the treatment seemed to be effective, a few weeks later she developed typical signs of pulmonary embolism which regressed spontaneously. She continued to take Waran® and has since felt well and had no symptoms of thromboembolism. Investigation revealed that AT III was low (45 % biological method, 37 % immunochemical method). All the other coagulation factors studied were normal, as were the platelets, the fibrinolytic components and the fibrinolytic activity in the vessel walls.

This patient had a large kindred and some relatives had had DVT. Four of the 23 members of the proband's generation II, besides herself, had had DVT. Moreover, one sister died at the age of 20 years from thrombosis and pulmonary embolism in the puerperium. In as many as 9 of the 18 examined members of generation II, AT III was significantly decreased.

All the members with a history of DVT had low AT III except a woman (II:6) who had once had DVT during pregnancy but a normal AT III level when examined about 25 years later. Five of the 9 persons with low AT III were paternal cousins. All of them denied any previous episode of DVT. A maternal cousin (II:1) had had DVT and his AT III was abnormally low on repeated occasions. Liver function tests showed nothing remarkable. Consanguinity was denied.

Twenty-six members of generation III were examined. Only 2 of them have so far had DVT, at 25 and 20 years of age, respectively. In one of them thrombosis had apparently been spontaneous and in the other it had occurred in the puerperium. In these 2 and in 7 others, AT III was low; in the remaining 17 members it was normal. Also 4 children of II:6 (with a history of DVT but normal AT III) were examined. AT III was normal in all of them. Thus, AT III was low in 18 of the 44 examined members of the family. Of the 18, four, all belonging to the maternal branch of the family, had had thromboembolism on at least one occasion. Repeated crossed immunoelectrophoresis of plasma from 9 members (II:1, 15, 17, 21; III:21, 24, 25, 27, 28) with low AT III showed a normal pattern, though the peaks were low compared with the mixed plasma (Fig. 2). As expected, crossed immunoelectrophoresis without heparin showed only one peak.

The correlation between the AT III values obtained by the biological assay and those determined immunochemically was high.

DISCUSSION

Since Egeberg (6) first published a family with a high incidence of thromboembolism associated with

decreased AT III activity, about 20 other families have been described. This paper reports a further family. Our finding that of 18 members with a significantly low AT III level, 6 had previously had thrombosis at least once, confirms the association between low AT III and predisposition to DVT. The heredity of AT III deficiency is autosomal dominant (6, 13, 15, 16). Our pedigree also fills all the criteria for autosomal dominant inheritance: (a) both sexes are affected equally often, (b) the trait appears in every generation, (c) the trait is transmitted by an affected person to half of his children (on average), and (d) unaffected persons do not transmit the trait to their children (25). Maternal and paternal relatives of our proband carried the abnormal gene, suggesting the recessive transmission. The heredity of AT III deficiency is, however, doubtlessly autosomal dominant because of the rarity of the condition in the general population.

AT III deficiency and DVT were more common on the mother's than the father's side. Actually none of the members on the father's side had had any signs of DVT, though AT III was low in 10 of them. This might be explained by a modifying gene counteracting the development of DVT. It might also be explained by some environmental factor predisposing the affected members of the maternal branch to thrombosis. In fact the *propositus* had her first attack of DVT in association with pneumonia, and her second in connection with childbirth. Further, one of the proband's sisters died from pulmonary embolism in the puerperium. The only person examined who had had DVT despite a normal AT III level developed it in the puerperium. Such predisposing factors were less common among the paternal members with low AT III. For example, 2 women with low AT III were never pregnant. Another factor is age: the members on the father's side were, on average, younger than on the mother's and therefore less predisposed to DVT. Egeberg (6) found that in the family described by him those members who had a low AT III level and developed thromboses did so in connection with trauma, surgery, infections or pregnancy. It should therefore be stressed that such factors aggravate the predisposition to thrombosis in persons with a low AT III level and that they should receive prophylactic treatment.

In all the members studied by us the correlation between the results of the biological and immunochemical methods was high. This means that none

of them had the type of AT III deficiency described by Sas et al. (23) and characterized by a normal amount of protein but with impaired function. There are obviously various forms of AT III deficiency, which underlines the importance of checking both the protein activity and protein related antigen.

When crossed immunoelectrophoresis with heparin (16.6 U/ml agarose) was introduced for investigation of AT III by Sas et al. (23), they found that 3 peaks appeared in normal plasma: a large fast one and 2 small slower ones. When heparin was not included in the first electrophoretic run, only a single high peak could be demonstrated. Examinations of our patients with decreased AT III showed a similar pattern with one high anodal peak followed by 2 lower ones. All the peaks were, however, lower than those in normal plasma. This contrasts with observations in the families investigated by Sas et al. (23) and Gomperts et al. (9), who found a low first peak migrating at a normal rate but a relatively larger second peak and no third peak. The pattern found in the family investigated by us implies a decreased synthesis of a normal protein as the cause of the low AT III levels.

Thus, several of the family members had low AT III, most probably due to a decreased synthesis of a normal protein. The genetic pattern is that of autosomal dominant inheritance. The low AT III level was very often associated with DVT. Additional environmental factors known to predispose to DVT seemed, however, to be important contributing causes of the thromboembolic episodes. It is therefore recommended that persons with a decreased AT III level should be given prophylactic treatment in any situation involving an increased risk of thrombosis, such as infections, surgery and other conditions requiring long bed-rest. Oral anticoagulants and/or dextran are recommended for such treatment since heparin does not produce the desired effect when AT III, which is identical with the heparin co-factor, is deficient.

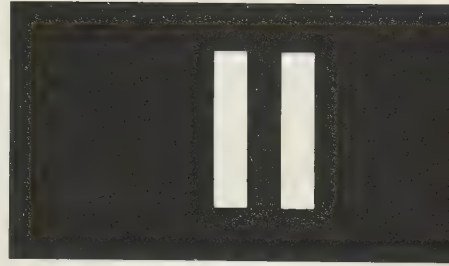
In acute DVT thrombolytic therapy might be considered. Oral anticoagulants alone or dextran could also be given. A satisfactory effect of heparin alone cannot be expected as AT III is the heparin co-factor, but it might be used combined with plasma to supply a certain amount of AT III. If acute DVT appears during pregnancy, dextran is recommended, since it does not enter the placenta, nor is it teratogenic as oral anticoagulants (8).

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REFERENCES

1. Abildgaard, U.: Highly purified antithrombin III with heparin cofactor activity prepared by disc electrophoresis. *Scand J Clin Lab Invest* 21:89, 1968.
2. Abildgaard, U., Fagerhol, M. K. & Egeberg, O.: Comparison of progressive antithrombin III activity and the concentrations of three thrombin inhibitors in human plasma. *Scand J Clin Lab Invest* 26:349, 1970.
3. Abildgaard, U., Fagerhol, M. K. & Godal, H. C.: Assay of progressive antithrombin III in plasma. *Thromb Diath Haemorrh* 24:224, 1970.
4. Carvalho, A. & Ellman, L.: Hereditary antithrombin III deficiency. Effect of antithrombin III on platelet function. *Am J Med* 61:179, 1976.
5. Cronberg, S.: Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets. With special reference to platelet adhesiveness. *Acta Med Scand (Suppl)* 486, 1968.
6. Egeberg, O.: Inherited antithrombin III deficiency causing thrombophilia. *Thromb Diath Haemorrh* 13:516, 1965.
7. Fagerhol, M. K. & Abildgaard, U.: Immunological studies on human antithrombin III. Influence of age, sex and use of oral contraceptives on serum concentration. *Scand J Haematol* 7:10, 1970.
8. Fourie, D. T. & Hay, I. T.: Warfarin as a possible teratogen. *S Afr Med J* 49:2081, 1975.
9. Gomperts, E. D., Feesey, M. & van der Walt, J. D.: Two dimensional immunoelectrophoretic studies in antithrombin III deficiency. *Thromb Res* 8:713, 1976.
10. Hedner, U. & Nilsson, I. M.: Antithrombin III in a clinical material. *Thromb Res* 3:631, 1973.
11. Hedner, U., Nilsson, I. M. & Jacobsen, C. D.: Demonstration of low concentration of fibrinolytic inhibitors in individuals with high fibrinolytic capacity. *Scand J Clin Lab Invest* 25:329, 1970.
12. Holmberg, L. & Nilsson, I. M.: Immunologic studies in haemophilia A. *Scand J Haematol* 10:12, 1973.
13. von Kaulla, E. & von Kaulla, K. N.: Deficiency of antithrombin III activity associated with hereditary thrombosis tendency. *J Med* 3:349, 1972.
14. Laurell, C. B.: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 15:45, 1966.
15. Marciniak, E., Farley, C. H. & de Simone, P. A.: Familial thrombosis due to antithrombin III deficiency. *Blood* 43:219, 1974.
16. van der Meer, J., Stoepman-van Dalen, E. A. & Jansen, J. M. S.: Antithrombin III deficiency in a Dutch family. *J Clin Pathol* 26:532, 1973.
17. Nilsson, I. M. & Olow, B.: Determination of fibrinogen and fibrinogenolytic activity. *Thromb Diath Haemorrh* 8:297, 1962.
18. — Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 123:247, 1962.
19. Owren, P. A. & Aas, K.: The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand J Clin Lab Invest* 3:201, 1951.
20. Pandolfi, M., Isacson, S. & Nilsson, I. M.: Low fibrinolytic activity in the walls of veins in patients with thrombosis. *Acta Med Scand* 186:1, 1969.
21. Robertson, B., Pandolfi, M. & Nilsson, I. M.: Response of local fibrinolytic activity to venous occlusion of arms and legs in healthy volunteers. *Acta Chir Scand* 138:437, 1972.
22. Sas, G., Blasko, G., Banhegyi, D., Jako, J. & Palos, L. A.: Abnormal antithrombin III (antithrombin III 'Budapest') as a cause of familial thrombophilia. *Thromb Diath Haemorrh* 32:105, 1974.
23. Sas, G., Pepper, D. S. & Cash, J. D.: Further investigations on antithrombin III in the plasmas of patients with the abnormality of 'antithrombin III Budapest'. *Thromb Diath Haemorrh* 33:564, 1975.
24. — Plasma and serum antithrombin-III: differentiation by crossed immunoelectrophoresis. *Thromb Res* 6:87, 1975.
25. Thompson, J. S. & Thompson, M. W.: Genetics in medicine, p. 38. Saunders, Philadelphia 1968.
26. Wolf, P.: A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. *J Clin Pathol* 6:34, 1953.



A Swedish family with abnormal antithrombin III

Lilian Tengborn¹, Birgitta Frohm¹, Lars-Erik Nilsson²
& Inga Marie Nilsson¹

¹Department for Coagulation Disorders and

²Department of Nuclear Medicine, University of Lund,
General Hospital, Malmö, Sweden

An abnormal variant of antithrombin III is reported in a young male with deep vein thrombosis. The heparin cofactor, progressive thrombin inhibition, and factor Xa inactivation are decreased. The abnormality seems to be a mutation which is transmitted in an autosomaldominant way. The half-life and fractional catabolic rate of 125 I antithrombin III concentrate is the same in this patient as in patients with the classic type of antithrombin III deficiency and in a control.

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Since Egeberg first reported a family with antithrombin III (AT III) deficiency in conjunction with thromboembolism (TE) in 1965 (1), several such families have been described. In most reported cases a parallel decrease has been found in AT III, as assayed both by immunochemical and functional methods. The first family with abnormal AT III in conjunction with TE was published by Sas et al (2); AT III Budapest, and later other types of abnormal AT III were described, see Table 1. Here we report the first Swedish family diagnosed with abnormal AT III, of a variant apparently similar to AT III Budapest.

Material and methods

Assays of coagulation system and vessel wall fibrinolysis

Activated partial thromboplastin time (APT time), coagulation factors (factor VIII coagulant activity, factor VIII related antigen, factor V, fibrinogen), thrombin and reptilase times, and tissue plasminogen activa-

tor activity after venous occlusion were analysed as described elsewhere (3). AT III was analysed (a) using electroimmunoassay (EIA) with antiserum raised in rabbits, prepared according to McKay (4), or antisera from Behringwerke (Marburg/Lahn, West Germany) and Nyegaard (Oslo, Norway); (b) as heparin cofactor activity, using the chromogenic substrate S-2238 (Kabi-Vitrum AB, Stockholm, Sweden) according to Ødegård et al (5); (c) as progressive AT III, using S-2238 according to Bauer et al (6); (d) as anti-Xa activity both in the presence and absence of heparin, as described by Ødegård et al (7), using the chromogenic substrate S-2222 (KabiVitrum AB, Stockholm, Sweden); (e) crossed immunoelectrophoresis (CIE) of plasma and serum according to Sas et al (8), using 20 IU heparin per ml agarose in the first dimension electrophoresis and antiserum prepared according to McKay (4) in the second dimension.

Catabolic studies

AT III concentrate was prepared by affinity chromatography on heparin Sepharose gel, according to Miller-Andersson et al (9), and was generously supplied by KabiVitrum AB (Stockholm, Sweden). The solution of

AT III was heat-treated for 10 h at 60°C in order to reduce the risk of hepatitis transmission.

Radio-labelling was done using McFarlane's iodine monochloride method (10) which, together with the working schedule, is described in detail elsewhere (11). The final amount of labelled AT III injected was 0.37–0.65 mg, corresponding to an activity of 1.13–1.50 MBq. The radioactivity of blood and urine samples was followed for 10 d. The trichloroacetic acid precipitate label in 1 ml plasma was counted at the same time as the radioactivity in 1 ml samples of urine. The plasma radioactivity values (%) were plotted on a semilog graph against time, the half-life being read from the final straight portion of the curve. The half-life was also calculated from the curve after being resolved into 3 exponential functions using the routine peeling-off technique.

Case report

A 29-year-old man who, for no apparent reason, developed a phlebographically-confirmed deep vein thrombosis (DVT) extending from the calf veins to the middle of the femoral vein. He was investigated at the Department for Coagulation Disorders after the acute incident, when he was otherwise in good health and had stopped prophylaxis with oral anticoagulants. Although there was no family history of TE, AT III was investigated in his parents, brother, sisters and his 4 children.

Catabolic studies using ^{125}I AT III concentrate were done on the propositus. As a comparison, such studies were also done on 1 control and on 2 patients with the classic type of AT III deficiency in whom both immunochemical and functional methods showed AT III about 50% of normal; 1 of these patients was reported on elsewhere (11). The study was approved by the Ethics Committee of the University of Lund.

Results

The results of analysis of APT time, coagulation factors, and the vessel wall fibrinolysis were normal. Table 2 shows the values obtained from the various AT III analyses. Repeated measurements using EIA remained consistently in the lower normal range, irrespective of which antisera were used. Values for heparin cofactor activity (S-2238), and for the anti-Xa activity (S-2222), both determined in the presence of heparin, were about 50% of the normal. In the absence of heparin, values for the progressive AT III and anti-Xa activity showed a slightly higher – although distinctly lower than the lower normal – limit.

CIE of normal plasma gave 1 high, fast moving peak, followed by 2 small, slow-moving peaks

TABLE 1
Variants of abnormal AT III previously reported

	Imm. chem.	S-2238 with heparin	S-2238 without heparin	Anti-Xa no heparin	Anti-Xa with heparin	CIE on plasma with heparin	Affinity chromatography with heparin Sepharose
Classical type	L	L	L	L	L	N	N
Abnormal types							
Budapest (2)	N	L	L	L	L	P	L
Basel (13)	N	L	N	N	L		
Paris (14)	N	L	N	N	L		L
Padua (15)	N	N				P	
Padua 2 (16)	N	L	N	N	L	P	N
Toyama (17)	H	L	N	N	L	P	0 (homozygote)
Ann Arbor (18)	N	L					
Aalborg (19)	N	L	L	N	N	N	N
Chicago (20)	N	L	L		L	N	H
Vicenza (21)	N	L				N (serum P)	
Milan (22)	N	L	L	L	L	N (without heparin P)	N
Aalborg 2 (23)	N	L	N	L	N	N	

L = low, N = normal, H = high, P = pathological.

TABLE 2

AT III determined by various assays. Values given are the means of repeated determinations

Family member	EIA	S-2238 with heparin	S-2238 without heparin	S-2222 with heparin	S-2222 without heparin
	%	%	%	%	%
Propositus	83	55	64	48	64
Father	98	108	122	105	108
Mother	89	92	109	101	104
Elder brother	17	105	115	110	106
Younger sisters	93	91	116	111	101
	104	111	120	113	104
Children	99	102	119	103	102
	99	96	115	99	108
	90	58	63	46	64
	86	50	71	50	68
Classic AT III deficiency	A 50	49	48	48	57
	B 47	45	51	46	56
Reference value range ^{a)}	75-120	80-125	97-106	95-110	96-107

x) The value is based on 20 healthy volunteers, 12 males, 8 females without contraceptive pills, mean age 36 years, range 17-60 years.

(Figure 1A). In the patients' plasma, the 1st peak was somewhat lower than in normal plasma and was followed by a single, rather high, slow-moving peak (Figure 1B).

CIE in normal serum gave 2 peaks, the 1st somewhat higher than the 2nd (Figure 1D); while the patients' serum gave a small, fast-moving peak and a rather high, slow-moving peak (Figure 1E). CIE performed on a mixture of normal plasma and patient plasma gave a 2nd peak with a position roughly corresponding to that of the 1st of the slow-moving peaks in normal plasma (Figure 1C).

The family investigation showed that both parents, the older brother and the younger sisters had completely normal values for all AT III assays (Table 2). 2 of the proband's 4 children, however, showed the same abnormality as their father.

The results of the catabolic studies of ¹²⁵I AT III concentrate are shown in Table 3. As can be seen, there was no difference in either half-life or fractional catabolic rate between the propositus, the subjects with classic AT III deficiency and the control.

Discussion

Against the background data of various reported abnormal variants of AT III (Table 1), the abnor-

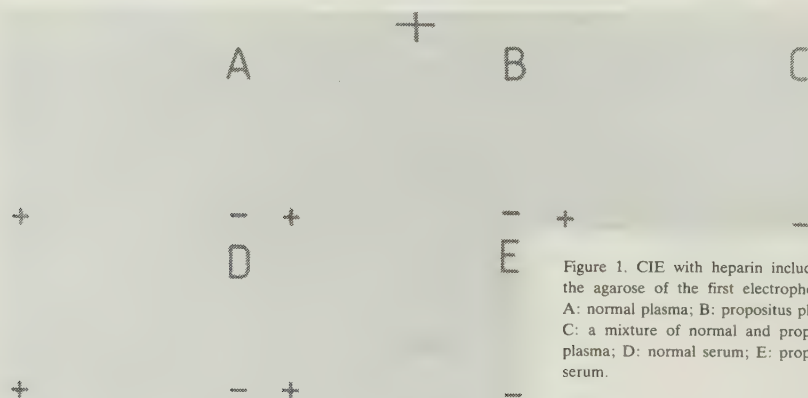


Figure 1. CIE with heparin included in the agarose of the first electrophoresis. A: normal plasma; B: propositus plasma; C: a mixture of normal and propositus plasma; D: normal serum; E: propositus serum.

TABLE 3

The variables, half-life and fractional catabolic rate, calculated from the results of studies using 125 I AT III concentrate in the propositus with abnormal AT III, 2 patients with the classic type of AT III deficiency, and 1 control

		Half-life days	FCR days ⁻¹
Propositus		2.9	0.61
Classic deficiency	A	3.0	0.71
	B	3.2	0.59
Control		2.9	0.64

mality in the family presented here seems to be largely the same in type as AT III Budapest, and belonging to a subgroup characterised by a fairly mild degree of abnormality (12). Thus, in our family, as in AT III Budapest, both thrombin and factor Xa were pathologically inactivated. Unlike several other families (AT III Aalborg, Basel, Paris, Padua 2, Toyama), our family showed a reduced heparin cofactor activity such as is found with AT III Budapest. Consanguinity was a feature in the AT III Budapest family, which probably explains the varying degrees of severity in that family. In our family, however, a mutation seems to have developed, the defective gene obviously being transmitted in an autosomal dominant way. In all 3 affected members of our family, apparently heterozygotes, laboratory results reflected the same mild degree of abnormality.

Since, in almost every report on abnormal AT III, association with DVT is described, it must be considered vital that suitable prophylaxis is given to patients with abnormal AT III in conditions known to predispose for TE. Thus, at operations and deliveries we feel the administration of AT III concentrate is emphatically indicated. As far as we know, no catabolic studies have hitherto been carried out in patients with abnormal AT III, and here we were able to demonstrate that the AT III concentrate was catabolised in the same way in our patient with abnormal AT III as in the 2 patients with the classic AT III deficiency and in the control.

Summing up, we consider that our family, with AT III abnormality and a general reduction of

various functions of the molecule, is closest in type to AT III Budapest.

Acknowledgements

We are indebted to Assistant Professor Bertil Nosslin for his invaluable advice and support of the pharmacokinetic studies.

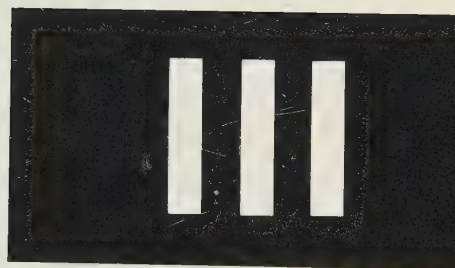
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References

1. Egeberg O. Inherited antithrombin III deficiency causing thrombophilia. *Thromb Diath Haemorrh* 1965;13:516-30.
2. Sas G, Blasko G, Banhegyi D, Jako J, Palos LA. Abnormal antithrombin III (antithrombin III "Budapest") as a cause of familial thrombophilia. *Thromb Diath Haemorrh* 1974;32:105-15.
3. Nilsson IM, Tengborn L. In: Jespersen J, Kluit C, Korsgaard O, eds. Impaired fibrinolysis. New evidence in relation to thrombosis. Esbjerg: South Jutland Press, 1983:173-91.
4. McKay EJ. A simple two-step procedure for the isolation of antithrombin III from biological fluids. *Thromb Res* 1981;21:375-82.
5. Ødegård OR, Lie M, Abildgaard U. Heparin cofactor activity measured with an amidolytic method. *Thromb Res* 1975;6:287-94.
6. Bauer KA, Teitel JM, Rosenberg RD. Assays for the quantitation of antithrombin III thrombin-antithrombin complex and prothrombin activation fragments. In: Colman RW, ed. *Disorder of thrombin formation*. New York: Churchill Livingstone, 1983:142-55.
7. Ødegård OR, Lie M, Abildgaard U. Antifactor Xa activity measured with amidolytic methods. *Haemostasis* 1976;5:265-75.
8. Sas G, Pepper DS, Cash JD. Plasma and serum antithrombin-III: differentiation by crossed immunoelectrophoresis. *Thromb Res* 1975;6:87-91.
9. Miller-Andersson M, Borg H, Andersson L-O. Purification of antithrombin III by affinity chromatography. *Thromb Res* 1974;5:439-52.
10. McFarlane AS. Efficient trace-labelling of proteins with iodine. *Nature* 1958;182:53.
11. Tengborn L, Frohm B, Nilsson LE, Nilsson IM. Antithrombin III concentrate: its catabolism in health and in antithrombin III deficiency. *Scand J Clin Lab Invest* 1981;41:469-77.
12. Sas G, Pető I, Bánhegyi D, Blasko G, Domján G. Heterogeneity of the "classical" antithrombin III deficiency. *Thromb Haemost* 1980;43:133-6.
13. Tran TH, Bondeli C, Marbet GA, Duckert F. Reactivity of a hereditary abnormal antithrombin III fraction in the inhibition of thrombin and factor Xa. *Thromb Haemost* 1980;44:92-5.
14. Wolf M, Boyer C, Lavergne JM, Larrieu MJ. A new familial

- variant of antithrombin III: "antithrombin III Paris". *Br J Haematol* 1982;51:285-95.
15. Girolami A, Pengo V, Cappellato G, Vianello C, Prociano M, Carlei C. Antithrombin III Padua: a "new" congenital antithrombin III abnormality with normal or near normal activity, normal antigen, abnormal migration and no thrombotic disease. *Folia Haematol Lip* 2 1983;110:98-111.
 16. Girolami A, Fabris F, Cappellato G, Sainati L, Boeri G. Antithrombin III (AT III) Padua 2. A "new" congenital abnormality with defective heparin cofactor activities but no thrombotic disease. *Blut* 1983;47:93-103.
 17. Sakuragawa N, Takahashi K, Kondo S, Koide T. Antithrombin III Toyama: a hereditary abnormal antithrombin III of a patient with recurrent thrombophlebitis. *Thromb Res* 1983; 31:305-17.
 18. Penner JA, Hassouna H, Hunter MJ, Chockley M. A clinically silent antithrombin III defect in an Ann Arbor family. VII International Congress on Thrombosis and Haemostasis, London 1970 (abstr 0437).
 19. Sørensen PJ, Sas G, Petó I, Blaskó G, Kremmer T, Samu A. Distinction of two pathologic antithrombin III molecules: antithrombin III 'Aalborg' and antithrombin III 'Budapest'. *Thromb Res* 1982;26:211-9.
 20. Bauer KA, Ashenurst JE, Chediak J, Rosenberg RD. Antithrombin 'Chicago': a functionally abnormal molecule with increased heparin affinity causing familial thrombophilia. *Blood* 1983;62:1242-50.
 21. Barbui T, Rodeghiero F. Hereditary dysfunctional antithrombin III (AT III Vicenza). *Thromb Haemost* 1981; 45:97.
 22. Boyer C, Tripod A, Wolf M, Mannucci PM, Larrieu MJ. A new familial variant of antithrombin III: antithrombin III Milano. *Thromb Haemost* 1983;50 (abstr 140).
 23. Mortensen JZ. Inherited AT - III deficiency - fast and slow inactivation of thrombin and factor Xa. *Thromb Res* 1984; 33:511-5.

Correspondence to:
Lilian Tengborn, M.D.
Department for Coagulation Disorders
Allmänna Sjukhuset
S-214 01 Malmö,
Sweden



Antithrombin III concentrate: its catabolism in health and in antithrombin III deficiency

LILIAN TENGBORN, BIRGITTA FRÖHM, L. E. NILSSON & INGA MARIE NILSSON

Coagulation Laboratory, University of Lund, Allmänna Sjukhuset, Malmö, Sweden

Tengborn, L., Fröhm, B., Nilsson, L.E. & Nilsson, I.M. Antithrombin III concentrate: its catabolism in health and in antithrombin III deficiency. *Scand. J. clin. Lab. Invest.* 41, 469-477, 1981.

The catabolism of purified radiolabelled antithrombin III (AT III) concentrate was studied in two normals and two patients with congenital AT III deficiency both alone and combined with warfarin. The radiolabelling with iodine monochloride did not change the quality of the concentrate. The half-life varied between 3.4 and 4.8 days. No difference between normals and patients with congenital deficiency in non-acute stage could be observed in the catabolic parameters; nor was there any influence with warfarin.

Key-words: AT III concentrate; AT III deficiency, half-life

Lilian Tengborn, M.D., Coagulation Laboratory, Allmänna Sjukhuset, S-214 01 Malmö, Sweden

The treatment of thromboembolic episodes in patients with congenital antithrombin III (AT III) deficiency has been the subject of much discussion. In the routine treatment with heparin and oral anticoagulants in the acute stage heparin is considered not to be sufficient because AT III is a heparin cofactor and required for the anticoagulant effect of heparin. Pregnant women especially constitute a problem since oral anticoagulants should not be given during pregnancy. But the situation has become brighter since the recent advent of AT III concentrate. This concentrate is now being tried out clinically and we have studied its effect in patients with congenital deficiency. In order to determine the half-life more exactly and to understand more fully the catabolism

of AT III we studied radiolabelled AT III concentrate as well. Several authors have reported an increase in AT III during medication with oral anticoagulants [13, 19, 22, 28]. In order to investigate possible effects of oral anticoagulants on AT III concentrate we also studied the catabolism with and without warfarin.

METHODS AND MATERIAL

Methods

Antithrombin III. AT III was determined (a) with the clotting method according to Abildgaard *et al.* [1] using heat defibrinated plasma (progressive AT III), (b) amidolytically with chromogenic substrate according to Ødegård *et al.* [23] using Coatest antithrombin

(S-2238) supplied by Kabi Diagnostica, Stockholm, Sweden, (c) electroimmunochemically (EIA) according to Hedner & Nilsson [11] using the rocket method of Laurell [15]. Mono-specific antiserum against AT III (batch No. 088) was obtained from Dr Johan Stenflo, Malmö, Sweden. The blood samples were diluted in 1/10 volume of 0.13 mol/l citrate. As reference we used pooled citrated plasma from 20 normals. This plasma contained 1 kU/l. The results were expressed in kU/l. The normal range for progressive AT III is 0.68–1.20 kU/l, for S-2238 0.92–1.18 kU/l and for EIA 0.74–1.21 kU/l.

Crossed immunoelectrophoresis. Carried out as described by Sas *et al.* [26] with mono-specific antiserum against AT III (batch No. 088) in the second dimension. Ninety $\times$ 180 mm plates were used with 25 ml of 1% agarose (Litex, Denmark) in electrophoresis buffer (sodium barbitone, 75 mmol/l, pH 8.6. Heparin (KabiVitrum, Stockholm, Sweden) in a concentration of 17 IU/ml agarose was incorporated in the first dimension gel.

Prothrombin and proconvertin. P & P (factor II + VII + X) was determined according to Owren & Aas [24]. Therapeutic range 7–15%.

AT III concentrate. Purified by KabiVitrum, Stockholm, Sweden, from human plasma by affinity chromatography on heparin-Sepharose gel. The method has been described in detail by Miller-Andersson *et al.* [20]. In the present investigations we used batches No. DcA 96, 99 and 104, all containing 500 U and 200 mg albumin per vial as indicated by the manufacturer. Each vial was reconstituted in 20 ml sterile water. The concentrates to be tested were diluted until their activities corresponded to those of the standard plasma and then tested in three dilutions. The activity of the concentrates was expressed in AT III kU/l (1 kU AT III is the amount of AT III activity present in 1 l standard plasma – 0.2 g).

Radiolabelling procedure. The following material was used; *albumin-free AT III concentrate* (batch No. DcA 107) containing 450 U per vial as indicated by the manufacturer; reconstituted in 18 ml sterile water. *Human serum albumin* (HSA), Albumin⁹⁰ 20%,

KabiVitrum, Stockholm, Sweden. *Iodine monochloride* solution prepared according to McFarlane [17]. Na^{125}I IMS 30, Radiochemical centre, Amersham, England (410–630 MBq/ μg I). *Trichloroacetic acid* (TCA) 30% solution in distilled water, E. Merck, Darmstadt, W. Germany.

Twenty-five units of AT III (mol.wt 67,000) according to Miller-Andersson *et al.* [20], was reacted with 0.5 nmol (35.5 MBq) Na^{125}I and 14.9 nmol ICl according to McFarlane [16] and Glover *et al.* [8]. The degree of iodination varied between 0.05 and 0.09 atoms of iodine per molecule AT III. Labelled AT III was purified on a Sephadex G-50 Fine column (16 $\times$ 2.5 cm) which was eluted with saline and the protein peak fractions were pooled. HSA was added in amounts equivalent to those in the albumin containing concentrate. The amount of non-TCA precipitable label after the sterile filtration varied between 1 and 2%. The final amount of AT III injected was 0.3×10^{-3} – 1.7×10^{-3} g with 0.5–1.3 MBq of ^{125}I .

Affinity chromatography. Performed on unlabelled and labelled AT III concentrate on heparin-Sepharose CL-6B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) according to the instructions of the manufacturer.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Performed in 10% gel according to Weber & Osborn [27].

Investigations with Azovan sodium (Evan's Blue, T-1824) in 0.1% solution [10]. The extinction was read spectrophotometrically (Beckman) in plasma at 620 nm. As control plasma drawn before the injection was used.

Analysis and calculations of the radioactive samples. The protein in 1 ml plasma was precipitated by addition of an equal volume of TCA and counted together with 1 ml samples of urine in an automatic well-type gamma counter (Wallac). The counts were corrected for background. The statistical counting error was less than 0.9%.

The plasma values were plotted in a semilog diagram with percentage of activity against time, and the half-time of each curve was read from its final straight part. The curves were graphically resolved into exponential functions according to the ordinary peeling-off technique. Each

curve could be adequately described as the sum of three exponential components. From the intercepts and slopes of the components, catabolism and distribution were calculated from the formulas used by Nosslin [21], in which the fractional catabolic rate (FCR) is expressed as fractions of plasma pool catabolized per day. The distribution is given as the ratio between total and intravascular plasma pool (T/P). The radioactivity of the urine samples was compared with that of simultaneous plasma values (U/P ratio) according to Campbell *et al.* [5] as a further check of the determination of the daily FCR.

Clinical material

Two patients (both males), 21 and 45 years of age, with known AT III deficiency and two healthy volunteers (also males), 28 and 32 years of age, with normal AT III, all in good health were studied. One of the patients (case I) has been reported earlier [12]. The AT III level was 0.39–0.55 kU/l as measured with the three methods. In the other patient (case II) the AT III was 0.51–0.62 kU/l as measured with the same methods. He belongs to a family with both AT III deficiency and a defective release mechanism of fibrinolytic activators from vessel walls. Apart from somewhat lower peaks the crossed immunoelectrophoretic patterns of the patients were normal. The first mentioned patient [on Warfarin (Waran®) about 2 years before this investigation] and the normals were examined both with and without Waran® medication; the second patient has been receiving continuous Waran® prophylaxis for several years because of recurrent pulmonary embolism. The P&P values were all regulated during the studies (5–30%). One hour before the injection of the radiolabelled material, and subsequently once a day, 0.3 g potassium iodide was given orally to avoid thyroid retention of metabolically released radioiodine. Case I and the normals received both a tracer dose of radiolabelled concentrate and a bulk dose of unlabelled concentrate simultaneously in every study, i.e. whether they were on Waran® or not. The amount of unlabelled concentrate was 1500 U for the normals and 2000 U for the patients. The second patient on permanent Waran® prophylaxis was first given a tracer

dose of radiolabelled AT III alone and then, in the second study, both radiolabelled and unlabelled concentrate. Blood samples were drawn before injection, repeatedly on the first day and thereafter twice a day, and later only once a day for 10–12 days. Urine samples were collected every 24 h throughout the observation period.

In one of the normals Azovan sodium was studied. Azovan sodium (10 ml of 0.1% solution) was mixed with 2.2 ml (1.2 mg) [¹²⁵I] AT III plus 7.8 ml (0.38 g) HSA. Immediately after the administration of the bulk dose of AT III concentrate the solution with Azovan sodium was injected. Blood samples were drawn before, 30 and 45 sec after the injection as well as after 3, 5, 10, 20, 30 min and 2 h. The quotients between extinction of the colour and the radioactivity counts were calculated.

RESULTS

In vitro assays of the AT III concentrate. The results of the studies using unlabelled AT III with HSA are shown in Table I. The EIA and clotting method showed the expected values of AT III, but the S-2238 was about 50% less than expected. Such unexpectedly low values were also found for the albumin-free AT III used for radiolabelling as well as for the radiolabelled AT III with added HSA (Table I). The crossed immunoelectrophoresis on unlabelled AT III with HSA, unlabelled AT III without HSA (before radiolabelling) and radiolabelled AT III with added HSA showed the same pattern with two peaks of roughly equal height (Fig. 1).

TABLE I. Assay of antigenic concentration and activity of the AT III concentrate used for infusion, the AT III concentrate without HSA for radiolabelling and the radiolabelled concentrate with HSA added

	Electro-immuno assay (kU/l)	Clotting assay (kU/l)	Amidolytic assay S-2238 (kU/l)
AT III conc. with HSA	1.04	0.99	0.54
AT III conc. without HSA	0.85	0.99	0.45
[¹²⁵ I] AT III conc. with HSA	0.86	0.93	0.45

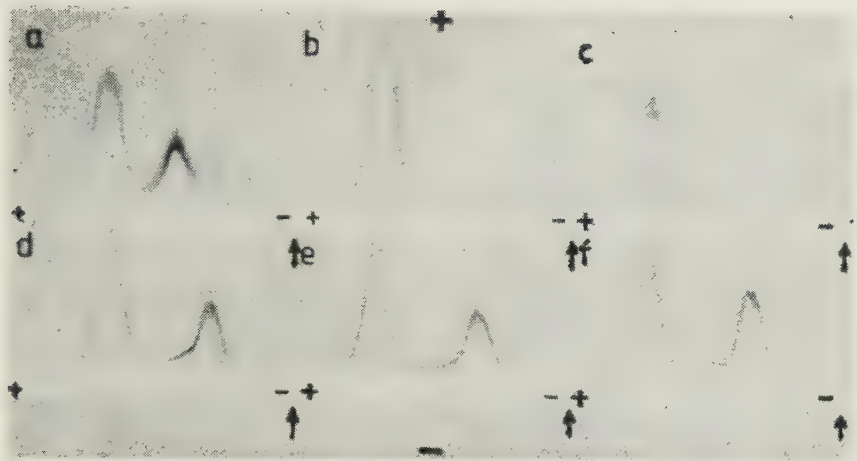


FIG. 1. Crossed immunoelectrophoresis of (a) normal serum, (b) normal plasma, (c) normal plasma diluted 1:2, (d) unlabelled AT III concentrate with HSA, (e) unlabelled AT III concentrate without HSA, (f) radiolabelled AT III concentrate with HSA. Arrow indicates application site.

In the figure the concentrates are compared with undiluted and diluted plasma as well as normal serum. When the same analysis was performed *without* heparin in the gel in normal serum, plasma and concentrate (unlabelled as well as labelled) there was, as expected [18], only one high single peak, since no separation of the free and different complex-bound AT III is obtained unless heparin is included. The AT

III concentrate studied with affinity chromatography showed that about half the amount of the unlabelled AT III was bound to the column and the other half was eliminated directly through the column without any signs of heparin-binding activity. The same result was obtained with the radiolabelled AT III. SDS-PAGE of unlabelled and labelled samples without HSA showed the same pattern with a

TABLE II. Calculated parameters in the controls and the cases with congenital AT III deficiency

	Plasma pool* (g)		(T/P)		FCR (days ⁻¹)		U/P (days ⁻¹)		Final T _{1/2} (days)		Rate of synthesis* (g/day)	
	-	+	-	+	-	+	-	+	-	+	-	+
Control I	0.710	0.592	2.0	2.1	0.53	0.50	0.61	0.66	3.9	3.8	0.376	0.296
II	0.605	0.605	2.8	2.3	0.57	0.40	0.46	0.51	4.3	4.8	0.345	0.242
Case I	0.320	0.288	2.2	2.0	0.52	0.45	0.65	0.56	3.7	3.4	0.166	0.130
II		0.360		2.4		0.54		0.62		3.8		0.194
II		0.354		2.3		0.51		0.59		3.7		0.181

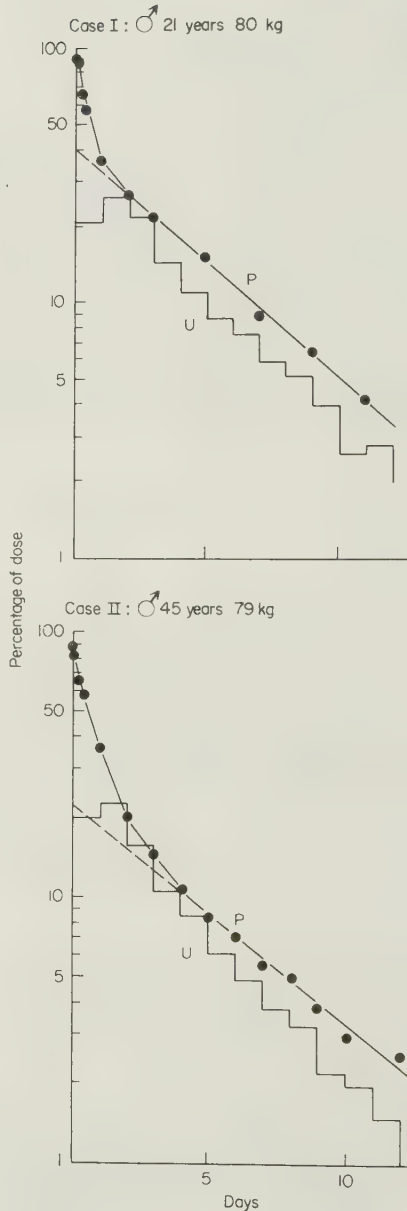
*Assuming a plasma volume of 0.04 g/kg body weight.
- and + denotes without and with Waran[®]. The controls and case I received both a tracer dose of labelled and a bulk dose on unlabelled AT III concentrate at the same time. Case II received, in the first study, labelled concentrate only and in the second both labelled and unlabelled concentrate. T/P = the ratio between total and plasma pool; FRC = fractional catabolic rate; U/P = the radioactivity in the urine samples compared with simultaneous plasma values.

single band with roughly the same molecular weight as albumin.

In vivo investigation. Azovan sodium was injected as a reference substance in order to find out whether any AT III was cleared from the blood within a few minutes as an inactivated substance. It is known that Azovan sodium is immediately bound to albumin and only 10% is eliminated during the first 2 h after injection [10]. If the simultaneously injected [125 I] AT III were eliminated, the extent of the elimination could be calculated as the quotient between radioactivity count and the concentration of Azovan sodium. The quotients were found to be constant for at least 2 h after the injection. This means that the AT III is not denatured; the radioactivity counts had rapidly decreased because, as well known, a denatured compound is cleared via the RES with a half-life of 5–10 min.

The calculated values for distribution and catabolism are given in Table II. The ratio of the total to the intravascular pool (T/P ratio) of AT III in the patients studied was 2.0–2.4 with warfarin and 2.2 without. The corresponding value for the controls were 2.1–2.3 and 2.0–2.8. The final half-life ($T_{1/2}$) values for the patients were 3.4–3.8 with, and 3.7 days without, warfarin. Those for the controls were 3.8–4.8 and 3.9–4.3 days, respectively. The rate of synthesis in the normals was about twice that in the patients with deficiency. The results of the radioactivity studies in the two patients are shown in Fig. 2. The determinations of AT III are given in Figs. 3 and 4. It is seen that the AT III level fluctuated somewhat in all the three methods and that warfarin has no effect on AT III in normals or patients with AT III deficiency.

FIG. 2. The plasma radioactivity (P) and the fractional daily urinary excretion of the label (U) in two cases with congenital AT III deficiency. Case I (with Waran®) received a bulk dose of 2000 U AT III concentrate. Case II (with Waran®) received no bulk dose.



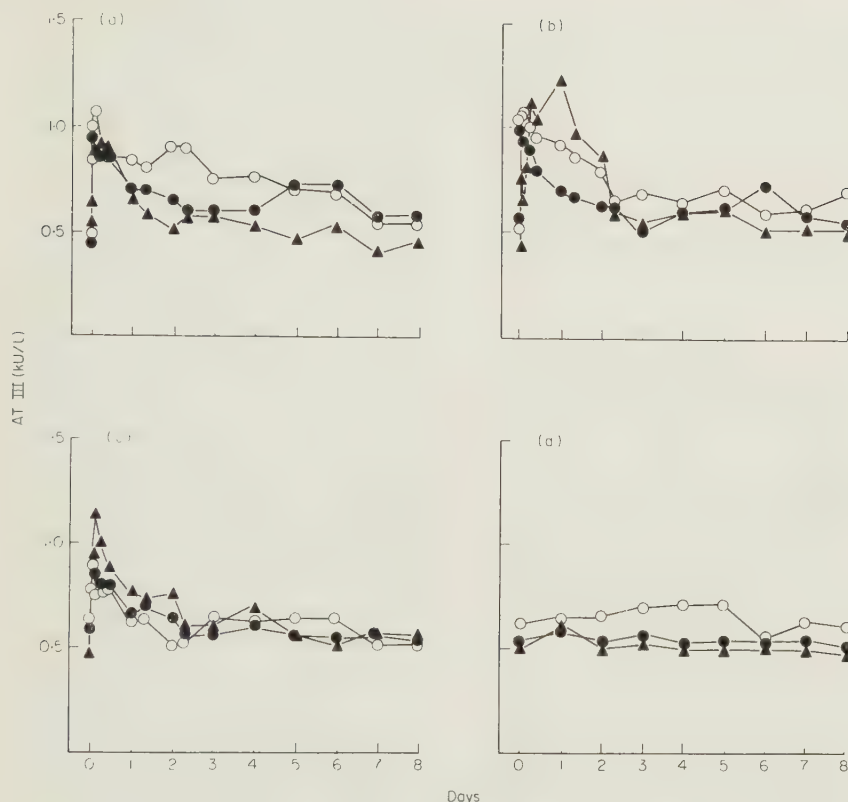


FIG. 3. The levels of AT III in the two cases with congenital AT III deficiency after infusion of a tracer dose of radiolabelled and a bulk dose (2000 U) of AT III concentrate (a, b and c). (d) Only tracer dose of radiolabelled concentrate was given; (a, c and d) on Waran®; (b) without Waran®. (a and b) Case I: male, 21 years, 80 kg. (c and d) Case II: male, 45 years, 79 kg. The following methods were used: EIA (●—●); amidolytic assay (○—○) and clotting assay (▲—▲).

DISCUSSION

Only few studies have been published on the catabolism of purified AT III. So far, only Collen and collaborators [6] have studied radiolabelled AT III. Collen and his group investigated normals, patients with arterial insufficiency, acute deep venous thrombosis with and without treatment with heparin and one patient with severe haemophilia A. Since no patient with congenital AT III deficiency has been studied with radiolabelled AT III we decided to investigate this group of patients.

Our studies were carried out with both

tracer doses of radiolabelled AT III and bulk doses of unlabelled concentrate. The reason why unlabelled concentrate was used was to increase AT III to such an extent that the changes could be followed with coagulation assays. The investigations in the second patient, who first received radiolabelled concentrate alone and later both radiolabelled and unlabelled concentrate at the same time (all the time on Waran®) did not show any difference in catabolism or distribution, which suggests that the bulk dose of AT III did not interfere with the catabolism.

The crucial points in the investigations

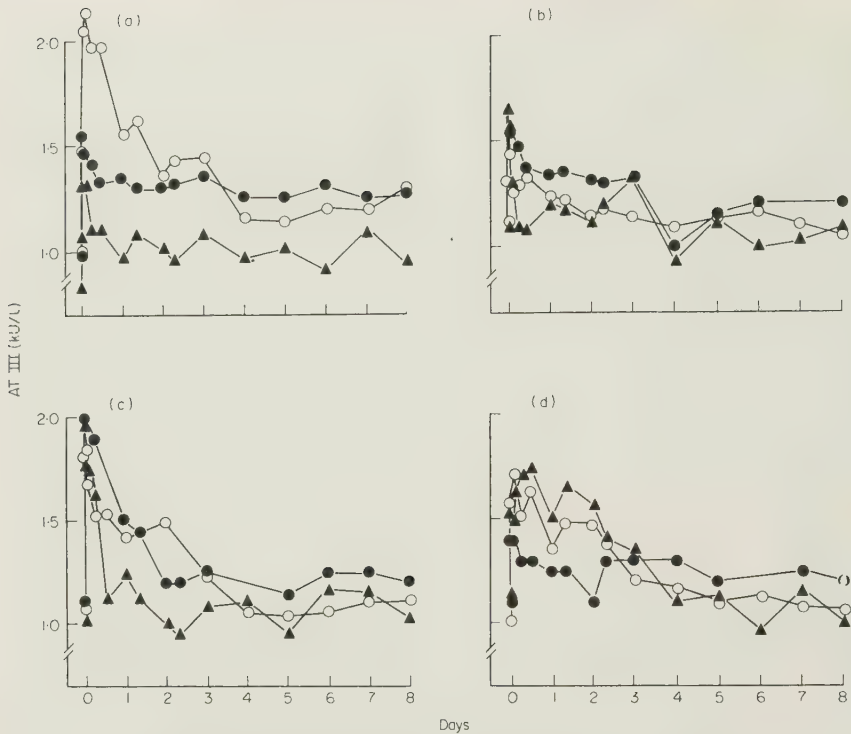


Fig. 4. The levels of AT III in the two controls after infusion of a tracer dose of radiolabelled and a bulk dose (1500 U) of AT III concentrate. (a and c) on Waran®; (b and d) without Waran®. (a and b) Control I: male, 32 years, 74 kg. (c and d) Control II: male, 28 years, 72 kg. The following methods were used: EIA (●—●); amidolytic assay (○—○) and clotting assay (▲—▲).

were the quality of the purified AT III concentrate as well as the quality of the concentrate after radiolabelling. A special preparation not containing HSA was used for radiolabelling. Studies of the different properties of the unlabelled and labelled concentrate showed that the radiolabelling procedure did not change the properties, as measured with the three coagulation assays, crossed immunoelectrophoresis, SDS-PAGE or affinity chromatography with heparin-Sepharose.

When the properties of the unlabelled AT III concentrate were assayed *in vitro* we found the expected values with the EIA (which determines the antigenic AT III protein) and by clotting assay (which determines the progressive activity). The amidolytic assay with heparin in the buffer which determines the heparin cofactor

activity, unexpectedly showed that the AT III level was decreased by about 50%. This difference between the two methods, both of which determine biological activity of AT III, might be explained by the fact that the assays measure two different functions. Such a difference was also found in the international collaborative study in 1977 [14].

It is known [3, 9, 12, 26] that the inclusion of heparin in the gel alters the immunoelectrophoretic pattern of AT III. Thus, in plasma a high fast-moving peak has been demonstrated and interpreted as a result of the rapid electrophoretic mobility of heparin, and the binding between heparin and AT III. In plasma this peak has been followed by small peaks consisting of non-heparin bound AT III. In serum two peaks of roughly equal height have been

observed. The AT III concentrate we used showed an electrophoretic pattern also with two peaks but the second peak in the concentrate migrated slower than the one in serum. These findings which are in agreement with others [3] suggest that the AT III molecule is changed to some extent at the heparin cofactor site. It has been suggested [2] that the pattern in the two dimensional electrophoresis might sometimes be an artefact due to the position of the sample being too close to the edge of the plate. In our experiments such artefacts can be excluded since we routinely place the sample at least 30 mm from the edge [12]. Thus, some inactivation of the heparin cofactor site probably occurred during the preparation. But the changes seem to be reversible bearing in mind the *in vivo* results after the infusion of the concentrate.

Taking all of our data, including coagulation assays, affinity chromatography and SDS-PAGE, we consider the AT III protein to be essentially native. This was also supported by the studies with Azovan sodium as well as by the calculated data about the catabolism.

Several authors have noticed that medication with oral anticoagulants increases the AT III [13, 19, 22, 28]. Others [7, 25] have found a decrease in AT III when such medication was withdrawn. It has been suggested that the increase in AT III on anticoagulants might be due to inhibition of a continuous low grade activation of the coagulation system. However, some authors [4] have not observed any significant increase in AT III on treatment with oral anticoagulants.

Our studies on AT III concentrate alone or combined with warfarin did not show any difference in the catabolism, distribution or half-life. Neither did the investigations by Collen *et al.* [6] with radiolabelled AT III lend support to the assumption of any existing consumption of coagulation factors, for they found a normal turnover rate in the patient who had severe haemophilia A.

Summing up, the most important findings were that there is no difference in catabolism or distribution of AT III between individuals with AT III deficiency and normals, and that its $T_{\frac{1}{2}}$ is 3.4–4.8 days. The synthesis rate in the normals is about twice that in individuals with AT III deficiency. The catabolism was not influenced by warfarin. From a clinical point of

view our studies indicate that although the heparin cofactor activity assessed in the concentrate seems to be affected, the AT III concentrate is suitable in treatment of AT III deficiency, also in combination with heparin.

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REFERENCES

- 1 Abildgaard, U., Fagerhol, M.K. & Egeberg, O. Comparison of progressive antithrombin activity and the concentrations of three thrombin inhibitors of human plasma. *Scand. J. clin. Lab. Invest.* **26**, 349, 1970.
- 2 Andersson, L.-O., Engman, L. & Henningsson, E. Crossed immunoelectrophoresis as applied to studies on complex formation. The binding of heparin to antithrombin III and the antithrombin III-thrombin complex. *J. Immun. Meth.* **14**, 171, 1977.
- 3 Barrowcliffe, T.W. Studies of heparin binding to antithrombin III by crossed immunoelectrophoresis. *Thrombos. Haemostas. (Stuttg.)* **42**, 1434, 1979.
- 4 Bull, H., MacKie, I.J., Brozović, M. & Woodings, D. Antithrombin III levels in patients on long term oral anticoagulants. *VII Int. Congr. on Thrombosis and Haemostasis*, 1979.
- 5 Campbell, R.M., Cuthbertson, D.P., Matthews, C.M. & McFarlane, A.S. Behaviour of ^{14}C - and ^{131}I -labelled plasma proteins in the rat. *Int. J. appl. Radiat.* **1**, 66, 1956.
- 6 Collen, D., Schetz, J., de Cock, F., Holmer, E. & Verstraete, M. Metabolism of antithrombin III (heparin cofactor) in man; effect of venous thrombosis and of heparin administration. *Eur. J. clin. Invest.* **7**, 27, 1977.
- 7 Filip, D.J., Eckstein, J.D. & Veltkamp, J.J. Hereditary antithrombin III deficiency and thromboembolic disease. *Am. J. Hemat.* **2**, 343, 1976.
- 8 Glover, J.S., Salter, D.N. & Shepherd, B.P. A study of some factors that influence the iodination of ox insulin. *Biochem. J.* **103**, 120, 1967.
- 9 Gomperts, E.D., Feese, M. & van der Walt, J.D. Two dimensional immunoelectrophoretic studies in antithrombin III deficiency. *Thromb. Res.* **8**, 713, 1976.
- 10 Gregersen, M.I. & Rawson, R.A. The disappearance of T-1824 and structurally related dyes from the blood stream. *Am. J. Physiol.* **138**, 698, 1943.
- 11 Hedner, U. & Nilsson, I.M. Antithrombin III in a clinical material. *Thromb. Res.* **3**, 631, 1973.
- 12 Johansson, L., Hedner, U. & Nilsson, I.M. Familial antithrombin III deficiency as pathogenesis of deep venous thrombosis. *Acta med. scand.* **204**, 491, 1978.
- 13 von Kaulla, E. & von Kaulla, K.N. Antithrombin III and diseases. *Am. J. clin. Path.* **48**, 69, 1967.
- 14 Kirkwood, T.B.L., Barrowcliffe, T.W. & Thomas, D.P. An international collaborative study establishing a reference preparation for antithrombin III. *Thrombos. Haemostas. (Stuttg.)* **43**, 10, 1980.

- 15 Laurell, C.B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analyt. Biochem.* **15**, 45, 1966.
- 16 McFarlane, A.S. Efficient trace-labelling of proteins with iodine. *Nature* **182**, 53, 1958.
- 17 McFarlane, A.S. Metabolism of plasma proteins. p. 297 in Munro, H.N. & Allison, J.B. (eds.) *Mammalian Protein Metabolism*. Academic Press, New York and London, 1964.
- 18 McKay, E.J. & Laurell, C.B. The interaction of heparin with plasma proteins. *J. Lab. clin. Med.* **95**, 69, 1980.
- 19 Marciniak, E., Farley, C.H. & de Simone, P.A. Familial thrombosis due to antithrombin deficiency. *Blood* **43**, 219, 1974.
- 20 Miller-Andersson, M., Borg, H. & Andersson, L.-O. Purification of antithrombin III by affinity chromatography. *Thromb. Res.* **5**, 439, 1974.
- 21 Nosslin, B. Mathematical model of plasma protein turnover determined with ¹³¹I-labelled protein. p. 115 in Andersen, S.B. (ed.) *Metabolism of Human Gamma Globulin (γ_{2S} globulin)*. Blackwell Scientific Publications, Oxford, 1964.
- 22 O'Brien, J.R. & Etherington, M.D. Effect of heparin and warfarin on antithrombin III. *Lancet* **ii**, 1232, 1977.
- 23 Ødegård, O.R., Lie, M. & Abildgaard, U. Heparin cofactor activity measured with an amidolytic method. *Thromb. Res.* **6**, 287, 1975.
- 24 Owren, P.A. & Aas, K. The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand. J. clin. Lab. Invest.* **3**, 201, 1951.
- 25 Refvem, O., Fagerhol, M.K. & Abildgaard, U. Changes in antithrombin III levels following cessation of anticoagulant therapy. *Acta med. scand.* **193**, 307, 1973.
- 26 Sas, G., Pepper, D.S. & Cash, J.D. Plasma and serum antithrombin-III: differentiation by crossed immunoelectrophoresis. *Thromb. Res.* **6**, 87, 1975.
- 27 Weber, K. & Osborn, M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. biol. Chem.* **244**, 4406, 1969.
- 28 Zucker, M.L., Gomperts, G.D. & Marcus, R.G. Prophylactic and therapeutic use of anticoagulants in inherited antithrombin III deficiency. *S. Afr. med. J.* **50**, 1743, 1976.

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IV

A Family with Thromboembolic Disease Associated with Deficient Fibrinolytic Activity in Vessel Wall

Lilian Johansson, Ulla Hedner and Inga Marie Nilsson

From the Coagulation Laboratory, Malmö General Hospital, Malmö, Sweden

ABSTRACT. Defective fibrinolytic activity is often a contributory factor in deep venous thrombosis. A family with a high incidence of venous thrombosis in association with such a defect is presented. Of 13 family members who had had thrombosis, 12 showed a defective capacity to release fibrinolytic activity from vessel wall after venous occlusion and/or infusion of DDAVP, a vasopressin derivate. The fibrinolytic activator activity of the vessel wall was normal in all cases. This seems to be the first family in which there is evidence of an inherited abnormal fibrinolytic activity.

Pathogenetic factors in the development of deep venous thrombosis (DVT) are disorders of components of the circulating blood and of the vessel wall. Thus, a selective increase of certain coagulation factors (6, 24) as well as of inhibitors of plasminogen activation (2, 18, 22) has been found in patients with a severe thromboembolic disease. An abnormally low level of antithrombin III is sometimes associated with an increased tendency to develop DVT (3, 5, 7, 14, 15, 16, 26). As for vessel wall changes predisposing to DVT, a decreased fibrinolytic activity in the endothelial cells, alone or combined with an impaired release of such activity, has been found in about 70% of all patients with recurrent thrombotic disease without any other known predisposing components (13). In some patients the connective tissue has an increased tendency to aggregate platelets (10).

This paper concerns the familial occurrence of thromboembolic disease associated with an impaired release capacity of fibrinolytic activity from the vessel wall.

PATIENTS AND METHODS

Seventeen members of the family were studied. All the tests were performed in a non-acute stage at least 4 weeks after an episode of DVT.

Laboratory methods

The following determinations were made: platelet count, platelet adhesiveness, prothrombin+proconvertin+factor X, factor V, factor VIII activity, factor VIII related antigen, fibrinogen, plasminogen, antithrombin III, α_2 -macroglobulin, fibrin/fibrinogen degradation products (FDP), inhibitors of the plasminogen activation, euglobulin clot lysis time and fibrinolytic activity of resuspended euglobulin precipitate of plasma on unheated fibrin plates. The procedures have been described elsewhere (4, 8, 11, 12, 19, 20, 21, 27). Fibrinolytic response to venous occlusion of the arms was assessed according to Robertson et al. (25). The fibrinolytic activity of resuspended euglobulin precipitate on unheated fibrin plates was determined after 20 min of venous occlusion. Human fibrinogen was used for the fibrin plates. The control group consisted of 52 apparently healthy volunteers. All persons investigated were examined on 2 consecutive days and afterwards at monthly intervals. 95% confidence interval 310-380 mm². Desamino-D-arginine-vasopressin (DDAVP) test was carried out as follows. DDAVP, 8 μ g in 100 ml saline, was administered as a 30-minute infusion. Blood samples were drawn before, immediately after, and 30 min after the infusion. Determinations were made of the fibrinolytic activity of resuspended euglobulin precipitates on fibrin plates. The control group consisted of 20 apparently healthy young males. Normal range before DDAVP infusion 40-160 mm², immediately after the infusion 140-320 mm², 30 min after the infusion 110-265 mm² (1).

Fibrinolytic activity in walls of superficial veins. A segment of a hand vein excised under local anaesthesia was examined with Pandolfi's modification of Todd's fibrinolysis autography technique by Pandolfi et al. (23). The activity was expressed in arbitrary units. The median value at our laboratory in 70 biopsy specimens of hand veins from healthy volunteers is 7.5 arbitrary units (range 6-10).

Family study

Fig. 1 shows the pedigree and Table I the results of investigations of 17 family members, 13 with a history of DVT.

The first member examined in the family was a boy (IV:26), born in 1959. In 1974, at the age of 14, he was admitted to hospital because of a swollen left leg.

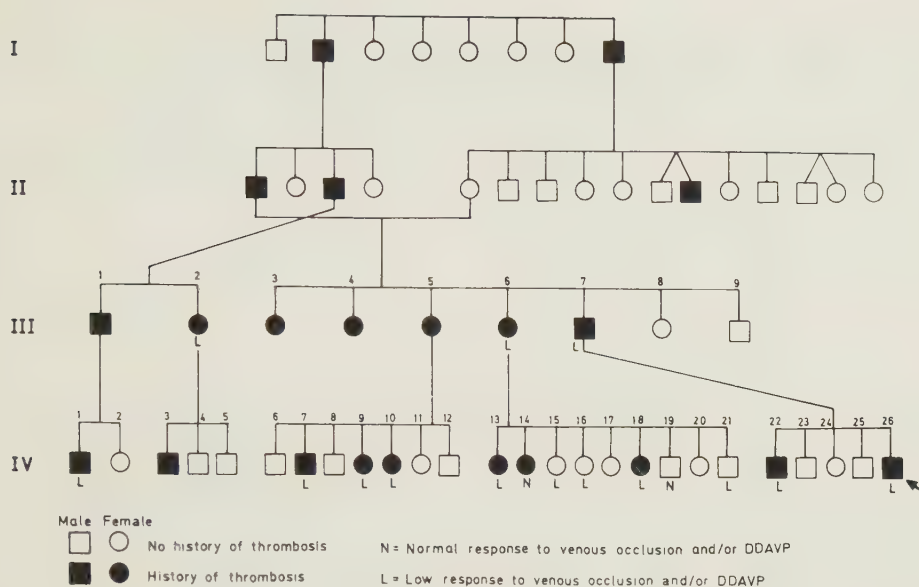


Fig. 1. Pedigree of the family ($\nearrow$ = proband).

Phlebography revealed thrombosis occluding the deep venous system, including the left iliac vein. Chest X-ray, pulmonary scintigram and angiography showed signs of bilateral embolism. Treatment was started with streptokinase and followed by heparin and dicoumarol. The boy recovered and was sent home with instructions to take dicoumarol regularly. So far, he has not shown any signs of a recurrence.

Laboratory studies revealed that the release of fibrinolytic activity from the vessel wall was deficient. The response to venous occlusion was abnormally weak, as was that to stimulation with DDAVP. The number and function of the platelets, the coagulation factors, plasminogen concentration, antithrombin III, inhibitors of plasminogen activators and α_2 -macroglobulin were all normal. Many of the patient's relatives had thromboembolism. The pedigree included a marriage between cousins.

The proband's elder brother (IV: 22), born in 1943, had an attack of DVT of unknown cause in 1971. Since then he has regularly taken dicoumarol and has not had any recurrence. It was found that he, too, had a defective release mechanism of fibrinolytic activity.

Among their 21 cousins and second cousins in generation IV, 8 had had episodes of DVT—6 on two or more occasions. IV: 3 had his first episode at 11 years of age and has since had DVT of one arm. Of 12 of the cousins and second cousins investigated, 9 proved to have an isolated defective fibrinolytic release mechanism after venous occlusion (6 with a history of DVT), and one (IV: 3 with first

DVT at 11 years) had such a defect at the first investigation as well as slightly elevated FDP and factor VIII activity. Later examinations revealed nothing remarkable.

The proband's father (III: 7) had had recurrent DVT in his twenties and thirties, most probably complicated by pulmonary embolism. Examinations revealed a defective release of fibrinolytic activity, but otherwise nothing remarkable. Moreover, 4 of his 6 siblings had had DVT. Only one of them was examined, an older sister with an earlier history of DVT. All her laboratory results were normal, except for a poor response of the fibrinolytic activity to DDAVP. Also 2 of the cousins in the same generation (III) had had DVT and one of them was available for examination. She responded poorly to venous occlusion but normally to DDAVP.

Thus, of the 17 family members investigated and included in Table I, 13 had had DVT. Of these 13 patients, the response of the fibrinolytic activity to venous occlusion and/or DDAVP was weak in 12. In 7 of them the response was poor in both tests, in 3 it was poor only after venous occlusion, and in one only in the DDAVP test. The remaining 2 patients were examined with only one of the tests. In one tested with venous occlusion the response was poor, and in the other the response to DDAVP was normal.

Of the 4 persons examined who had had no episode of DVT in their history, one responded normally to venous occlusion (DDAVP was not performed), one normally to DDAVP but poorly to venous occlusion, one poorly to both tests, and one poorly only to venous occlusion.

Table I. The fibrinolytic activity of resuspended euglobulin precipitate on fibrin plates after venous occlusion (normal $>310 \text{ mm}^2$) and immediately after DDAVP infusion (normal $>140 \text{ mm}^2$) and the fibrinolytic activity in biopsy specimens in superficial veins (normal >6 arbitrary units)

Generation no.	Venous occlusion, mm^2		DDAVP (mm^2)	Biopsy (arbitrary units)	No. of DVT
	Day 1	Day 2			
III: 2	500	286	131	—	>2
III: 6	403	343	75	7.0	>2
III: 7	392	127	113	—	>2
IV: 1	396	59	—	8.75	>2
IV: 3	399	84	64	—	>2
	383			8.25	
	493	282			
IV: 7	344	270	136	7	2
	415				
IV: 9	201	126	90	7.0	1
IV: 10	390	102	182	9.5	1
					(Superficial thrombophlebitis)
IV: 13	320	115	110	9.0	>2
IV: 14	428	359	—	10.25	>2
IV: 15	424	237	—	9.75	0
IV: 16	245	240	81	—	0
IV: 18	401	254	165	9.0	2
IV: 19	328	331	—	8.0	0
IV: 21	259	277	214	9.25	0
IV: 22	323	109	69	6.5	1
IV: 26	106	88	38	6.5	1

DISCUSSION

In about 70% of their patients with spontaneous, recurrent DVT, Isacson and Nilsson (13) found the fibrinolytic activity in the vessel wall and/or the capacity to release such activity into the circulating blood to be decreased after venous occlusion. Many of the members of the family presented in this paper had had DVT. We investigated 13 members with and 4 without DVT in their histories. Of the 13 patients found to have had DVT, 12 repeatedly showed a defective capacity to release fibrinolytic activity from the vessel wall after venous occlusion and/or DDAVP infusion. The fibrinolytic activator activity of the vessel wall was normal in all.

Only 2 of the 11 patients with decreased fibrinolytic activity after venous occlusion had low values on both days they were investigated. The remaining 9 showed a normal fibrinolytic activity at the first examination but, unlike normals, a significantly low value at the second. This indicates an abnormally early exhaustion of the fibrinolytic release capacity. The findings also stress how important it is to assess the fibrinolytic capacity on 2 consecutive days. One of the family members with repeated attacks of

DVT in his history showed a defective release capacity at the first examination as well as mild reactive signs with elevated factor VIII activity and FDP. On later occasions the laboratory values were normal. This might indicate that the release capacity may vary from time to time, perhaps in association with various forms of reactive processes. IV: 14, who had had DVT but showed normal fibrinolytic activity after venous occlusion (DDAVP test was not performed), had her first attack of DVT shortly after delivery. Since then she had had several relapses of DVT of unknown cause.

Combined ethyloestrenol and phenformin can normalize the fibrinolytic capacity of a defective vessel wall. Nilsson et al. (17) found that normalization of the capacity tended to reduce the frequency of the thromboembolic episodes in 75 patients treated for periods of 3–48 months. A phenformin-like substance is being tried as a single drug (9). In the family presented in this paper, 7 members, all with the special release defect described, are receiving treatment with this drug. It is still too early to evaluate the effect.

It appears that this is the first family with evi-

dence of heredity of an increased frequency of DVT in association with a defective release capacity of fibrinolytic activity from the vessel wall. We therefore feel it important to examine all young persons who have had one or more attacks of DVT of unknown cause, and in selected cases to extend the investigations to include relatives.

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REFERENCES

1. Åberg, M. & Nilsson, I. M.: Plasminogen activator release after venous occlusion and infusion of DDAVP. VI Int. Congr. on Thrombosis and Haemostasis, p. 298. Philadelphia 1977.
2. Brakman, P., Mohler, E. R. & Astrup, T.: A group of patients with impaired plasma fibrinolytic system and a selective inhibition of tissue activator-induced fibrinolysis. *Scand J Haematol* 3: 389, 1966.
3. Carvalho, A. & Ellman, L.: Hereditary antithrombin III deficiency. Effect of antithrombin III deficiency on platelet function. *Am J Med* 61: 179, 1976.
4. Cronberg, S.: Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets. With special reference to platelet adhesiveness. *Acta Med Scand (Suppl)* 486, 1968.
5. Egeberg, O.: Inherited antithrombin III deficiency causing thrombophilia. *Thromb Diath Haemorrh* 13: 516, 1965.
6. Gaston, L. W.: Studies on a family with an elevated level of factor V (proaccelerin) and a tendency to thrombosis. *J Pediatr* 68: 367, 1966.
7. Gomperts, E. D., Feesey, M. & van der Walt, J. D.: Two dimensional immunoelectrophoretic studies in anti-thrombin III deficiency. *Thromb Res* 8: 713, 1976.
8. Hedner, U. & Nilsson, I. M.: Antithrombin III in a clinical material. *Thromb Res* 3: 631, 1973.
9. — Effect of moroxydine chloride on fibrinolysis in the vessel wall. Third Int. Conf. on Synthetic Fibrinolytic Thrombolytic Agents—Progr. in Fibrinolysis, Glasgow 1976.
10. Hedner, U., Nilsson, I. M., Bergentz, S.-E. & Cronberg, L.: Hyperactive connective tissue in seven patients with recurrent thrombotic occlusions. *Haemostasis* 1: 48, 1973.
11. Hedner, U., Nilsson, I. M. & Jacobsen, C. D.: Demonstration of low content of fibrinolytic inhibitors in individuals with high fibrinolytic capacity. *Scand J Clin Lab Invest* 25: 329, 1970.
12. Holmberg, L. & Nilsson, I. M.: Immunologic studies in haemophilia A. *Scand J Haematol* 10: 12, 1973.
13. Isacson, S. & Nilsson, I. M.: Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. *Acta Chir Scand* 138: 313, 1972.
14. von Kaulla, K. N. & von Kaulla, E.: Antithrombin III and diseases. *Am J Clin Pathol* 48: 69, 1967.
15. Marciniak, E., Farley, C. H. & de Simone, P. A.: Familial thrombosis due to antithrombin III deficiency. *Blood* 43: 219, 1974.
16. van der Meer, J., Stoepman-van Dalen, E. A. & Jansen, J. M. S.: Antithrombin III deficiency in a Dutch family. *J Clin Pathol* 26: 532, 1973.
17. Nilsson, I. M., Hedner, U. & Isacson, S.: Phenformin and ethyloestrenol in recurrent venous thrombosis. *Acta Med Scand* 198: 107, 1975.
18. Nilsson, I. M., Krook, H., Sternby, N.-H., Söderberg, E. & Söderström, N.: Severe thrombotic disease in a young man with bone marrow and skeletal changes and with a high content of an inhibitor in the fibrinolytic system. *Acta Med Scand* 169: 323, 1961.
19. Nilsson, I. M. & Olow, B.: Determination of fibrinogen and fibrinogenolytic activity. *Thromb Diath Haemorrh* 8: 297, 1962.
20. — Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 123: 247, 1962.
21. Owren, P. A. & Aas, K.: The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand J Clin Lab Invest* 3: 201, 1951.
22. Pandolfi, M., Hedner, U. & Nilsson, I. M.: Bilateral occlusion of the retinal veins in a patient with inhibition of fibrinolysis. *Ann Ophthalmol* 2: 481, 1970.
23. Pandolfi, M., Isacson, S. & Nilsson, I. M.: Low fibrinolytic activity in the walls of veins in patients with thrombosis. *Acta Med Scand* 186: 1, 1969.
24. Penick, G. D., Dejanov, I. I., Reddick, R. L. & Roberts, H. R.: Predisposition to intravascular coagulation. *Thromb Diath Haemorrh (Suppl)* 21: 543, 1966.
25. Robertson, B., Pandolfi, M. & Nilsson, I. M.: Response of local fibrinolytic activity to venous occlusion of arms and legs in healthy volunteers. *Acta Chir Scand* 138: 437, 1972.
26. Sas, G., Blasko, G., Banhegyi, D., Jako, J. & Palos, L. A.: Abnormal antithrombin III (antithrombin III 'Budapest') as a cause of familial thrombophilia. *Thromb Diath Haemorrh* 32: 105, 1974.
27. Wolf, P.: A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. *J Clin Pathol* 6: 34, 1953.



IMPAIRED FIBRINOLYSIS. NEW EVIDENCE IN
RELATION TO THROMBOSIS

I.M. Nilsson, L. Tengborn

Department for Coagulation Disorders, University of Lund,
Allmänna Sjukhuset, Malmö, Sweden

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Editors

Jørgen Jespersen

Section of Coagulation and Fibrinolysis, Department of
Clinical Chemistry, Ribe County Hospital in Esbjerg,
and Section of Thrombosis Research, University Centre
of South Jutland, Esbjerg, Denmark.

Cornelis Kluft

Gaubius Institute, Health Research Division TNO,
Leiden, The Netherlands.

Ove Korsgaard

Department of Clinical Chemistry, Ribe County Hospital
in Esbjerg, Esbjerg, Denmark.

South Jutland University Press, Esbjerg, Denmark, 1983

Key words: thrombosis
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 t-PA
 t-PA inhibitor

Correspondence: Dr Lilian Tengborn
 Department for Coagulation Disorders
 Allmänna Sjukhuset
 S-214 01 Malmö
 Sweden

INTRODUCTION

In 1972 Isacson and Nilsson (1) reported that patients with venous thrombosis had a high frequency of defective fibrinolysis. Thus, among 117 patients with "idiopathic" recurrent deep venous thrombosis (DVT) or thrombophlebitis studied in remission 73 % had a decreased fibrinolytic activity in the vein walls and/or a defective fibrinolytic activity after venous occlusion. Later the material was enlarged and the tendency was the same (2). Johansson et al (3) reported on a family with high incidence of DVT associated with deficient fibrinolytic activity after venous occlusion. Other families with reduced fibrinolytic activity and thrombotic disease have also been reported (4, 5, 6).

The aim of the present study was to investigate the fibrinolytic activity in the family reported by us (3) with thrombotic disease and decreased fibrinolytic activity by recently developed methods in order to get further knowledge about the abnormality.

METHODS

APT time, fibrinogen, thrombin time, reptilase time, fibrinolytic activity of resuspended euglobulin precipitate on fibrin plates and inhibitors of plasminogen activation were assayed as described earlier (7). Plasminogen was determined by radial immunodiffusion (own antiserum) and by amidolytical method using S-2251. Antithrombin III (AT III) was assayed (a) by electroimmunoassay using own antiserum; (b) heparin cofactor activity using S-2238. Protein C was assayed by a semiquantitative immunochemical method as described by von Schwinck et al (8) using own antiserum.

Fibrinolytic activity in the vein walls (biopsy specimen from superficial hand vein) was determined by Pandolfi's modification of Todd's fibrinolysis autography technique. The cut section was covered with a fibrin film rich in plasminogen. The activity was expressed in arbitrary units according to Pandolfi et al (9).

Quenching of fibrinolytic activity was performed according to Ljungnér et al (10). Plasminogen activator of tissue like type was obtained from a melanoma cell line (kindly provided by Dr D. Collen) and purified as described by Wallén et al (11). Antisera against the melanoma cell activator and against urokinase (UK) was raised in goats (12, 13), and the IgG of these antisera was isolated. An IgG preparation from normal goat was used as control.

Fibrinolytic response to venous occlusion (20 minutes' occlusion) of the arms was assessed according to Robertson et al (14). Resuspended euglobulin precipitate was analysed on fibrin plates (15). Human fibrinogen was used. The test was performed on two consecutive days. The lower limit of 95 % confidence interval in apparently healthy volunteers was 310 mm² (3).

Tissue plasminogen activator (t-PA) related substance was analysed by immunoradiometric assay (IRMA; M:Ag) as described by Holmberg et al (13). Purified antibodies to melanoma activator was prepared as described above and radio-labelled with ¹²⁵I by lactoperoxidase method. The assay was performed as two-side solid phase assay in polystyrene tubes. The sensitivity of the assay was calculated to 0.7 µg/l.

t-PA was determined amidolytically according to Gyzander et al (16). The t-PA and plasminogen in the sample were absorbed by lysine-Sepharose. The lysine-Sepharose was then mixed with glu-plasminogen, poly-D-lysine and S-2251 and the absorbance was read. For the two t-PA methods melanoma activator was used as reference and the activity expressed as ng/ml.

t-PA and its inhibitor was studied using SDS-polyacrylamide gel electrophoresis (PAGE) followed by zymographic analysis according to Philips and Thorsen (17).

CLINICAL MATERIAL

A. Members of the family earlier described (3)(Fig. 1). Two brothers were further studied, IV:22 and IV:26. The propositus, IV:26, now 24 years of age, developed at 14 without any known reason DVT phlebographically confirmed in the left leg extending to the iliac vein. He also had pulmonary embolism confirmed by angiography. Number IV:22, 40 years, developed DVT of unknown reason at the age of 28.

B. Apparently healthy individuals (8 males, 7 females, mean age 33 years, range 19-53).

RESULTS

As can be seen from Fig. 1 thrombosis has appeared in five generations. Altogether 33 members in 3 generations have so far been examined. They had normal values for APT time, fibrinogen, thrombin time, reptilase time, plasminogen, antithrombin III and protein C. Biopsy vein walls examined by autography technique showed normal values.

The fibrinolytic response to venous occlusion, however, was defective. In generation III only 3 thrombotic patients were analysed out of whom 2 had reduced fibrinolytic activity after the occlusion test. In generation IV all 10 patients who had had thrombosis were examined. All except one had a defective fibrinolytic activity. Moreover, another five persons between 21 and 39 years of age, had a defective fibrinolytic activity but hitherto no thrombosis.

In generation V 13 members were studied. Six had a defective fibrinolytic activity but until now no thrombosis. Four of them were below 20 years of age. Two patients, who had had thrombosis, one spontaneously and one postoperatively, had unexpectedly normal fibrinolytic activity.

In Fig. 2 the fibrinolytic response to venous occlusion on two consecutive days in the family members with and without thrombotic disorder is shown. In most of the patients the defective fibrinolysis was demonstrated only on the second day of examination. In the normal material no substantial difference in response to day 1 and day 2 was registered. The fibrinolytic activity was quite stable among these patients as can be seen in Fig. 3 showing 2 patients (IV:22 and IV:26) studied on 8 different occasions in the cause of 8 and 9 years, respectively.

Thus, numbers IV:22 and IV:26, were further investigated. Firstly specimens from vein walls were examined by histochemical technique using monospecific antibodies against the melanoma activator and UK as well as control IgG in the fibrin films. It was demonstrated that the fibrinolytic activity was quenched by the antibodies against melanoma activator but it remained unchanged when anti-UK and control IgG were used.

Secondly, the fibrinolytic activity emerging during venous occlusion was studied by three different methods. Figure 4 shows the results after venous occlusion on two consecutive days. In the normal individuals there was a marked increase of the activity as measured by all three methods and no obvious difference between day 1 and day 2. In the two patients the t-PA related substance measured by IRMA increased to the same levels as seen in the normal material. On the contrary, using the fibrin plate method and the amidolytic method the patients were found to have a substantially reduced activity. Thus, the antigen levels of the patients ranged between 14 and 17 ng/ml, while the amidolytic assay gave results of less than 0.3 ng/ml.

Furthermore, purified melanoma activator in varying concentrations was added to normal plasma and to plasma from the patients drawn before venous occlusion, and after incubation for 15 min at 37°C the residual t-PA activity was measured by the amidolytic method (Table 1).

The plasma of the patients had a markedly increased inhibitor activity. On day 1 the plasma of the younger brother (IV:26) inactivated 5 times more activator than normal plasma, and on day 2, i.e. 24 h after venous occlusion of both

arms, his plasma showed about 50 times higher inhibitor activity than normal plasma did. The older brother, too, had an increased inhibitor activity.

Using SDS-PAGE, followed by zymographic analysis a band with activity of M_r about 95,000 could be demonstrated both in post-occlusion plasma from the patients and from normal individuals (Fig. 5a). Various amounts of purified melanoma activator was added to pre-occlusion plasma. After addition of 40 ng activator to 1 ml of normal plasma a band with marked activity was seen in the region of M_r 70,000 and only a minor band of activity at the 95,000 M_r region. On the other hand, in plasma from the patients no activity was seen in the 70,000 M_r region but a major band of activity in the 95,000 M_r region (Fig. 5b).

DISCUSSION

Since we in 1978 (3) described a family with high incidence of vein thrombosis associated with decreased fibrinolytic activity of the vessel wall as measured after venous occlusion on fibrin plates several new methods for assessment of fibrinolysis have been developed. The patients in this family had a normal fibrinolytic activity analysed by a histochemical method using fibrinolysis autography technique of biopsy specimen. Reinvestigation using the same histochemical method but including monospecific antibodies in the fibrin film revealed that the activity in the vessel wall was immunologically identical with the tissue like plasminogen activator derived from melanoma activator. No quenching could be discovered using anti-UK or normal IgG.

Of interest in the reinvestigated patients was the finding, that after venous occlusion these patients released normal levels of activator measured by IRMA but markedly decreased values by biological methods. This discrepancy indicates that the defect might be due to a release of an abnormal activator with reduced capacity to bind to fibrin or it might be due to the activator being inactivated by an inhibitor. Loskutoff and coworkers (18) have recently demonstrated that endothelial cells in bovine aorta synthesize a fibrinolytic inhibitor that modulates the fibrinolytic activity of the vessel wall. In endothelial cell cultivations from human umbilical cord veins Philips and Thorsen (17) as well as Levin and Harker (19) found, using SDS-PAGE technique followed by zymographic detection, t-PA activity and anti-t-PA in a complex formation with M_r about 95,000 and 100,000, respectively. Chmielewska et al (20) found evidence for the presence of a fast inhibitor to t-PA in low and variable concentrations in plasma. Using SDS-PAGE followed by a zymographic analysis Kruithof et al (21) found that M_r of the complex t-PA and antiactivator was about 110,000 when investigating human plasma. When we performed SDS-PAGE and zymographic technique on post-occlusion plasma, we did not find any difference in the complex between t-PA and its supposed inhibitor in the patients and the controls indicating that the complexes have similar molecular weights. After addition of purified melanoma activator to plasma it was clearly demonstrated that in the patients all the activator was complexed indicating that the inhibitor in the patients had a higher capacity to bind to activator as compared to the normal controls.

The investigations of this family suggested that the defect of the vessel wall fibrinolysis consists of an increased production and/or release of t-PA like inhibitor. However, we consider it odd that an inherited defect should be due to an increased synthesis of inhibitor. One possibility is that these patients have an abnormal inhibitor with increased affinity for the t-PA or that the recorded increased inhibitor activity is only a step in a more complex regulation system. The fact that the inhibitor activity was higher on day 2 than on day 1 may indicate that inhibitor had been released from the endothelial cells following venous occlusion on day 1. Attempts are now being made to identify both the released t-PA and the inhibitor in our patients.

To sum up, when studying two members from a family where thrombosis had occurred in five generations a normal release of t-PA was demonstrated after venous occlusion as measured by IRMA but a decreased fibrinolytic activity according to biological methods. In preocclusion plasma the thrombotic patients

15. Nilsson IM, Olow B. Fibrinolysis induced by streptokinase in man. *Acta Chir Scand* 1962;123:247-66.
16. Gyzander E, Eriksson E, Teger-Nilsson AC. Determination of tissue plasminogen activator in plasma after adsorption of lysine-sepharose. In: Bachmann F, Bouvier CA, Kruithof EKO. eds. Sixth International Congress on Fibrinolysis, Lausanne, July 1982, p 68 (abstract).
17. Philips M, Thorsen S. Binding of plasminogen activator (PA) to a component (X) of endothelial cells (EC) may explain the 95,000-M_r PA in human plasma. IX International Congress on Thrombosis and Haemostasis, Stockholm 1983, p 193 (abstract).
18. Loskutoff DJ, van Mourik JA, Erickson LA et al. Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. IX International Congress on Thrombosis and Haemostasis, Stockholm 1983, p 194 (abstract).
19. Levin EG, Harker LA. An inactive complex of tissue plasminogen activator and inhibitor produced by human endothelial cells in culture. IX International Congress on Thrombosis and Haemostasis, Stockholm 1983, p 194 (abstract).
20. Chmielewska J, Rånby M, Wiman B. Determination of tissue plasminogen activator in plasma. Evidence for a rapid inhibitor. IX International Congress on Thrombosis and Haemostasis, Stockholm 1983, p 193 (abstract).
21. Kruithof EKO, Ransijn A, Tran-Thang F et al. Characteristics of a fast acting inhibitor of plasminogen activator in human plasma. IX International Congress on Thrombosis and Haemostasis, Stockholm 1983, p 193 (abstract).

Table 1. Residual t-PA after addition of various amounts of melanoma activator (MA) as assayed by amidolytic method (N = number)

Added amount of MA ng/ml	Residual MA (ng/ml)							
	Normals		day 1		day 2		IV:22	
	N	mean	day 1 range	N	mean	day 2 range	day 1	day 2
10	8	3.2	0-6.3	14	3.0	0-11	<1	0
50	8	28.4	5.1-39	14	28.8	11-65	25	0
100	4		62-100				45	21
200	4		144-200				132	50
500	4		~500				~500	~500
							~500	~500
							day 1	day 2
							IV:26	
							day 1	day 2
							0	0
							0	0
							31	0
							78	0
							~500	18

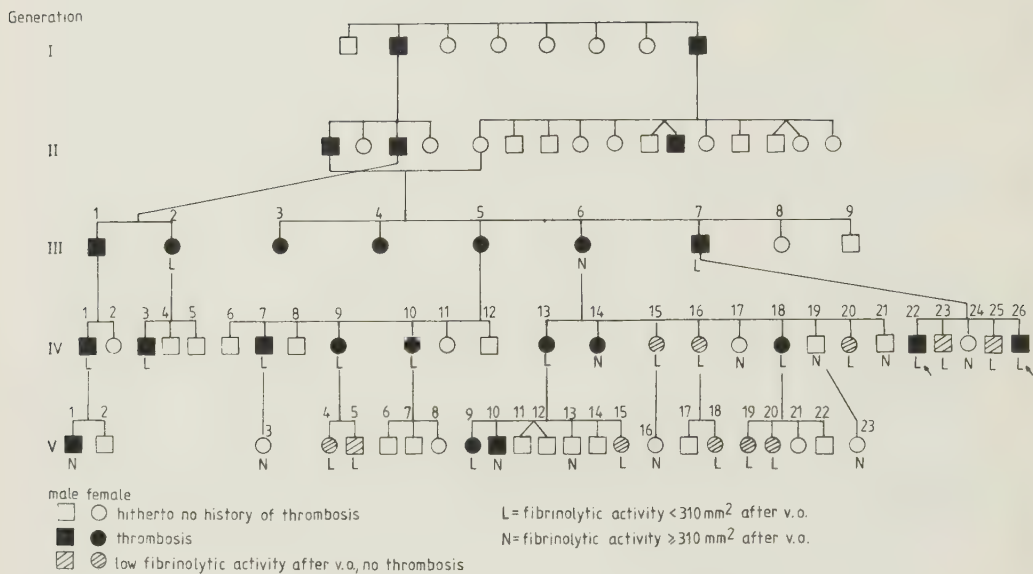


Fig. 1 Pedigree of the family. ➤ generation IV no. 22 and 26 were reinvestigated by recently developed assays.

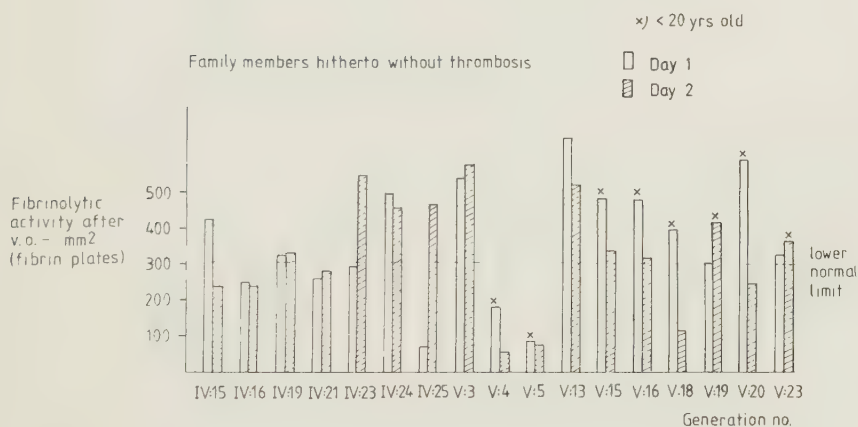
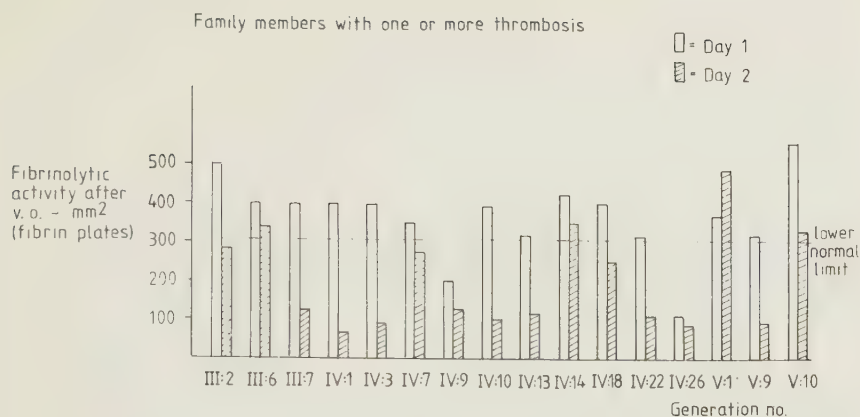


Fig. 2 Fibrinolytic activity on fibrin plates after venous occlusion of family members a) with DVT and b) hitherto without DVT. Dashed line shows the lower limit of 95 % confidence interval in normals. Decreased fibrinolytic activity as in several cases demonstrated only on the second day of examination.

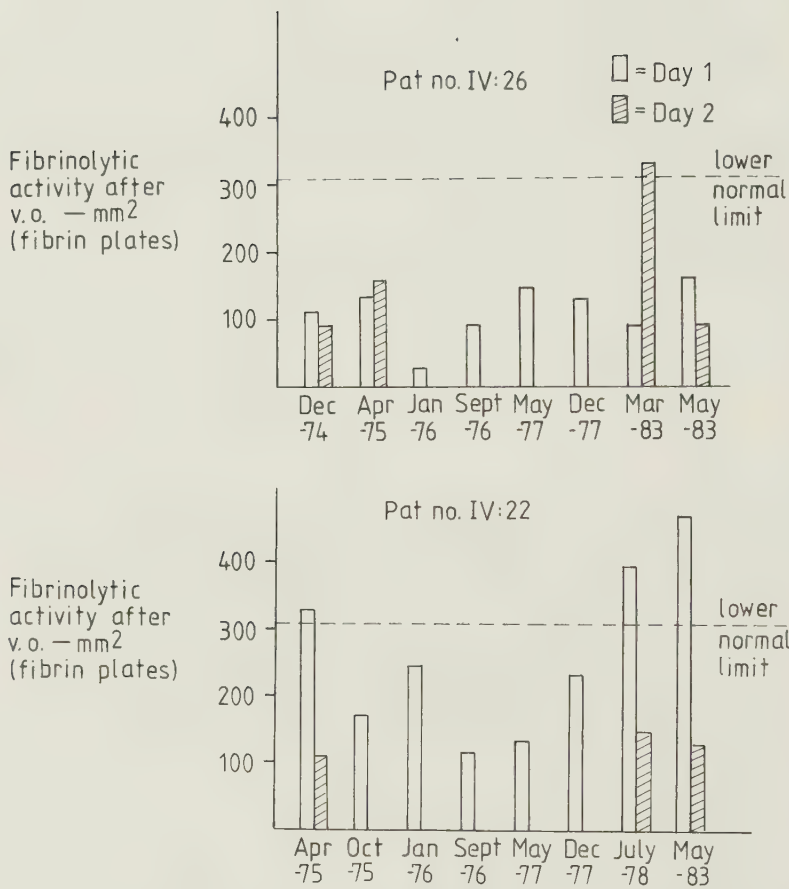


Fig. 3 Repeated investigations of fibrinolytic activity on fibrin plates of patients no. IV:22 and IV:26.

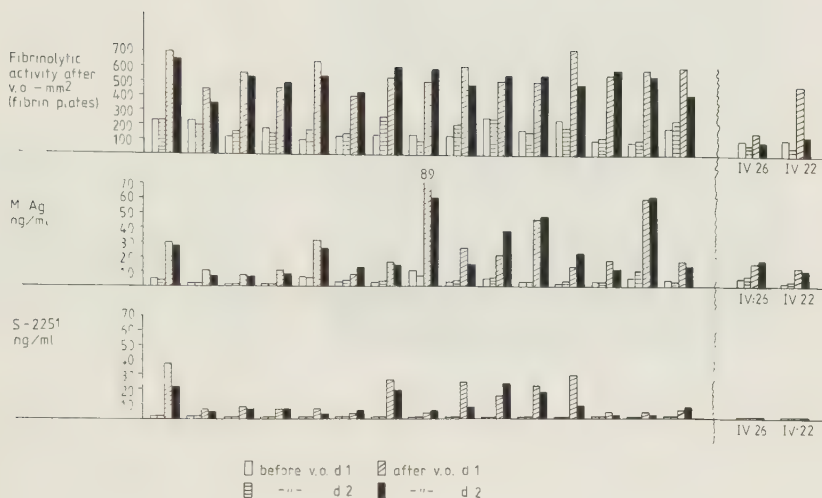


Fig. 4 Determinations by three different assays (see Methods) before and after venous occlusion on two consecutive days.

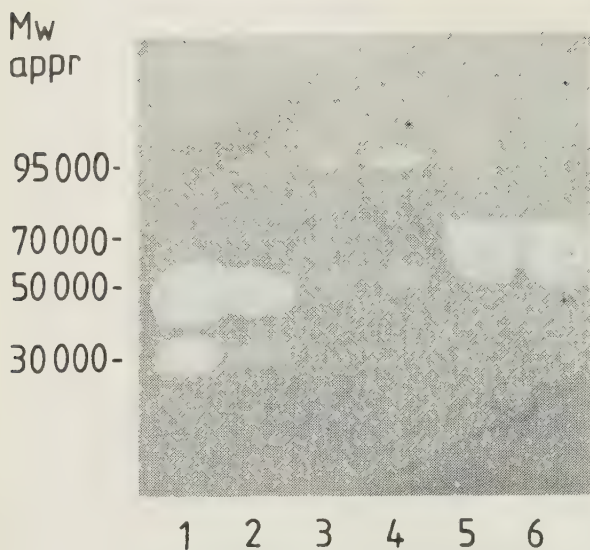
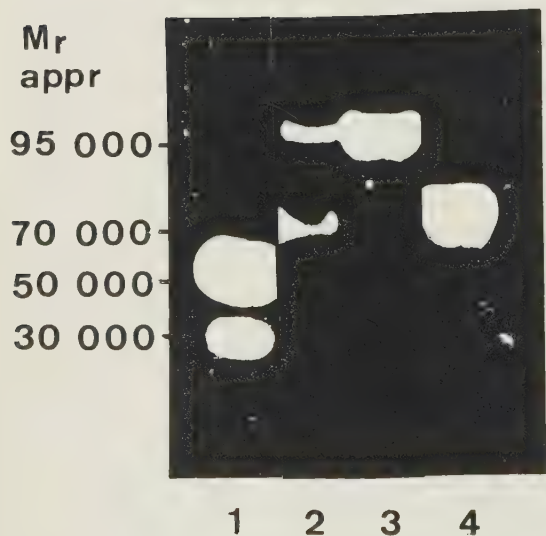


Fig. 5

a

SDS-PAGE followed by zymographic analysis of urokinase 0.5 and 0.25 PU (1 and 2) citrated plasma from patient no. IV:26 (3) and normal plasma (4) after venous occlusion diluted 1:10, melanoma activator 6.5 and 3.25 ng/ml (5, 6).



b

Urokinase 0.25 PU (1), melanoma activator 3.25 ng/ml (4), addition of melanoma activator 40 ng/ml to pre-occlusion plasma diluted 1:10 of normal person (2) and patient no. IV:26 (3).

